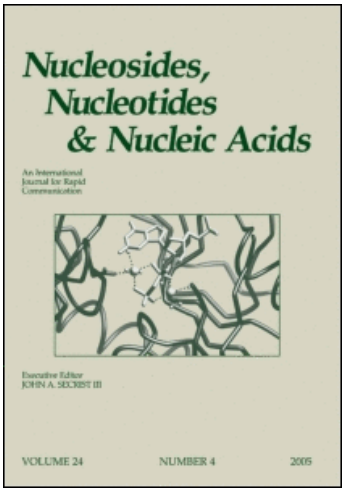


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis, Conformation of 3'-(Tetrazole-2''-yl)-3'-deoxythymidine and its 5''-Derivatives. Substrate Properties of 3'-(Tetrazole-2''-yl)-3'-deoxythymidine 5'-Triphosphate

V. A. Ostrovskii[a]; E. P. Studentsov[a]; V. S. Poplavskii[a]; N. V. Ivanova[a]; G. V. Gurskaya[b]; V. E. Zavodnik[c]; M. V. Jasko[b]; D. G. Semizarov[b]; A. A. Krayevsky[b]
[a] St-Petersburg Technological Institute, St-Petersburg [b] Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia [c] Karpov Physicochemical Research Institute, Moscow, Russia

To cite this Article Ostrovskii, V. A. , Studentsov, E. P. , Poplavskii, V. S. , Ivanova, N. V. , Gurskaya, G. V. , Zavodnik, V. E. , Jasko, M. V. , Semizarov, D. G. and Krayevsky, A. A.(1995) 'Synthesis, Conformation of 3'-(Tetrazole-2''-yl)-3'-deoxythymidine and its 5''-Derivatives. Substrate Properties of 3'-(Tetrazole-2''-yl)-3'-deoxythymidine 5'-Triphosphate', Nucleosides, Nucleotides and Nucleic Acids, 14: 6, 1289 — 1300
To link to this Article: DOI: 10.1080/15257779508010691
URL: http://dx.doi.org/10.1080/15257779508010691

NUCLEOSIDES & NUCLEOTIDES, 14(6), 1289-1300 (1995)

SYNTHESIS, CONFORMATION OF 3'-(TETRAZOLE-2"-YL)-3'-DEOXYTHYMIDINE AND ITS 5"-DERIVATIVES. SUBSTRATE PROPERTIES OF 3'-(TETRAZOLE-2"-YL)-3'-DEOXYTHYMIDINE 5'-TRIPHOSPHATE

V.A. Ostrovskii, E.P. Studentsov, V.S. Poplavskii, N.V. Ivanova, G.V. Gurskaya†, V.E. Zavodnik#, M.V. Jasko†, D.G. Semizarov†, A.A. Krayevsky†x

St-Petersburg Technological Institute, 26, Moskovskii Pr., St-Petersburg 198013; †Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32, Vavilov Str., Moscow 117984, Russia; #Karpov Physicochemical Research Institute, 10, Obukha Str., Moscow 103064, Russia

Abstract: 5'-O-Benzoyl-3'-(tetrazole-2"-yl)-3'-deoxythymidine and its 5"-substituted derivatives were obtained by the reaction of 5'-O-benzoyl-2,3'-anhydrothymidine with triethylammonium salts of either tetrazole or 5-substituted tetrazoles. Debenzoylation of these compounds yielded 3'-(tetrazole-2"-yl)-3'-deoxythymidine and its 5"-derivatives. Structures of two of them were confirmed by X-ray analysis. Both 3'-(tetrazole-2"-yl)-3'-deoxythymidine and 3'-(5"-methyltetrazole-2"-yl)-3'-deoxythymidine have *anti*-conformation with respect to the glycosidic bond, and 2'-*endo*-3'-*exo*-conformation of the sugar residue with *gauche*$^+$ orientation relative to the C4'-C5' bond. 3'-(Tetrazole-2"-yl)-3'-deoxythymidine 5'-triphosphate exhibited poor termination substrate properties towards avian myeloblastosis virus reverse transcriptase and did not serve as a substrate for other employed DNA polymerases.

Abbreviations used: HIV - human immunodeficiency virus type 1; AMV - avian myeloblastosis virus; KFr - DNA polymerase I Klenow fragment from *E. coli*.

---

x To whom correspondence should be addressed

At present 3'-modified 2',3'-dideoxynucleosides such as 3'-azido-3'-deoxythymidine, 2',3'-dideoxyinosine, 2',3'-dideoxycytidine, and 2',3'-deoxy-2',3'-didehydrothymidine are used as anti-AIDS agents [1-3]. However, these compounds are quite toxic and for this reason different laboratories continue to search for 3'-substituted 2',3'-dideoxynucleosides exhibiting anti-AIDS activity. In this connection, such 3'-azido-3'-deoxythymidine analogs as 3'-azole derivatives are rather interesting. A series of similar compounds containing 3'-N-pyrrolyl- and 3'-N-pyrazolyl- residues, have been synthesized. Among them are 3'-N-(1",3",4"-triazolyle)-3'-deoxythymidine, 3'-N-(1",2",3"-triazolyle)-, 3'-N-(1",2",4"-triazolyle)-3'-deoxyarabinothymidines [4], as well as 3'-N-(5"-alkyl- or arylaminotetrazole-1"-yl)-3'-deoxythymidines [5]. They all were obtained either by formation of the corresponding azole cycle on the basis of the amino group of 3'-amino-3'-deoxythymidine or by the reaction of the corresponding azole with 2',3'-lyxoanhydrothymidine and subsequent removal of the 2'-hydroxyl.

Results and Discussion

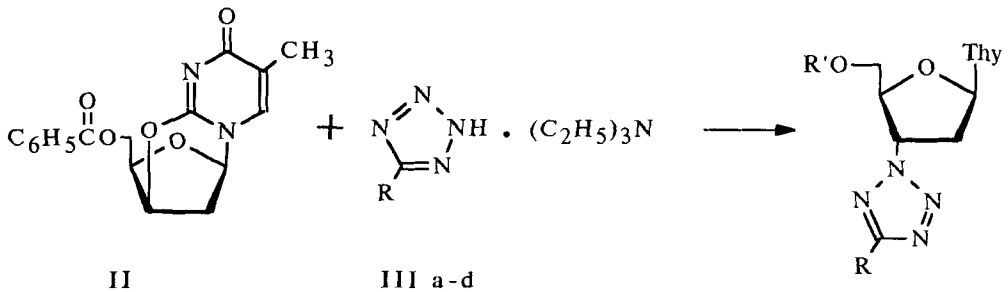
In this paper we showed that 3'-N-(tetrazole-2"-yl)-3'-deoxythymidine **Ia** and its 5"-derivatives **Ia-Id** can be obtained by the interaction of 5'-O-benzoyl-2,3'-anhydrothymidine **II** with triethylammonium salts of tetrazole and tetrazole 5-derivatives **IIIa-IIId** in DMF-dioxane (Scheme I).

Ammonium salts of tetrazole and tetrazole 5-derivatives can exist in aprotic dipolar solvents as complexes with hydrogen bonds (mono-, di-, and trialkylammonium salts) or dissociate with the formation of solvated ions (quaternary ammonium salts). These salts are used for preparation of tetrazole N-derivatives of different structures [6]. One should take into account possible formation of isomeric N1- and N2- derivatives of tetrazole at the ratio, determined by their electron properties at the tetrazole C5 atom as well as by steric factors [7].

Purity and structure of the synthesized compounds were confirmed by elemental analysis, NMR, and mass-spectrometry (Tables 1-3). The results of X-ray analyses of **Ia** and **Ib** proved the structure of these compounds.

Figure 1 presents the molecule **Ia** and **Ib** X-ray structures and atomic numbering accepted in this work. The atomic coordinates of the non-hydrogen atoms and their thermal parameters are listed in Table 4.

It was shown that molecules of **Ia** and **Ib** have similar dimensions and conformations. In the both compounds, the *anti* orientation of thymine relative



II III a-d

IV a-d R'= C_6H_5CO

I a-d R'= H

Va R'= $H_4O_9P_3$

R = H (a), CH_3 (b), C_6H_5 (c), $C_6H_5CH_2$ (d)

Scheme I

Table 1. Characteristics of the obtained compounds

№	m.p. °C	R_f	molecular formula	elemental analysis						yield, %	mass spectra
				found, %			calculated, %				
				C	H	N	C	H	N		
Ia	170-172	0.26	$C_{11}H_{14}N_6O_4$	44.88	5.06	28.61	44.90	4.80	28.55	37	294
Ib	205-207	0.22	$C_{12}H_{16}N_6O_4$	46.85	4.98	27.48	46.75	5.23	27.25	45	308
Ic	204-207	0.40	$C_{17}H_{20}N_6O_4$	55.56	4.47	23.23	55.13	4.90	22.69	61	370
Id	134-137	0.39	$C_{18}H_{18}N_6O_4$	56.56	4.81	22.24	56.24	5.24	21.86	20	384
IVa	182-185	0.50	$C_{18}H_{18}N_6O_5$	53.55	4.65	21.55	54.27	4.55	21.09	30	398
IVb	159-162	0.46	$C_{19}H_{20}N_6O_5$	55.20	4.95	20.49	55.34	4.88	20.38	51	412
IVc	218-220	0.64	$C_{24}H_{22}N_6O_5$	60.29	4.34	17.29	60.75	4.67	17.71	32	474
IVd	113-121*	0.63	$C_{25}H_{24}N_6O_5$	61.68	5.12	17.42	61.47	4.95	17.20	44	488

* - with decomposition

Table 2. ^{1}H NMR spectra of compounds **Ia-d** and **IVa-d** [DMSO-d_6, δ, ppm (*J*, Hz)]

№	H-6 s	H-1' t (7.5)	H-2' m	H-3' m	H-4' m	H-5' m	CH_3 s	NH s	others
Ia	7.80	6.55	2.7-2.9	5.75	4.35	3.80	1.70	11.40	5.35t (5) OH, 9.10s H-5"
Ib	7.80	6.50	2.7-2.9	5.65	4.25	3.70	1.80	11.35	2.50s CH_3, 5.35t (5) OH
Ic	7.90	6.65	2.7-2.9	5.8	4.40	4.00	1.85	11.35	7.65m Ph
Id	7.80	6.30	2.7-2.8	5.65	4.3	3.70	1.80	11.00	2.10m PhCH_2, 7.3m Ph
IVa	7.80	6.60	3.00	6.15	4.70		1.70	11.60	8.15m Ph, 9.3c H-5"
IVb	7.65	6.60	3.10	6.00	4.70		1.70	11.20	2.50c CH_3, 8.15m Bz
IVc	7.75	6.65	3.00	6.15	4.78		1.70	11.60	8.15m Ph+Bz
IVd	7.35	6.65	2.95	6.00	4.65		1.65	11.60	4.3m PhCH_2, 7.65m Ph, 8.05m Bz

Table 3. ^{13}C NMR spectra of compounds **Ia-d** (DMSO-d_6, δ, ppm)

№	C-2	C-4	C-5	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	C-5"	$5CH_3$	others
Ia	150.5	163.7	109.9	136.1	84.1	36.3	62.9	83.9	61.4	153.7	12.3	—
Ib	150.5	163.8	109.9	136.1	84.1	36.2	62.7	84.0	61.1	162.8	12.3	10.63, 5"CH_3
Ic	150.6	163.8	109.9	136.2	84.2	36.3	63.2	83.9	61.2	164.5	12.3	126.5, 126.8, 129.4, 130.8, Ph
Id	150.5	163.8	109.9	136.9	84.1	36.2	62.9	83.9	61.1	165.4	12.3	31.0, CH_2Ph; 126.8, 128.6, 128.8, 136.1, Ph

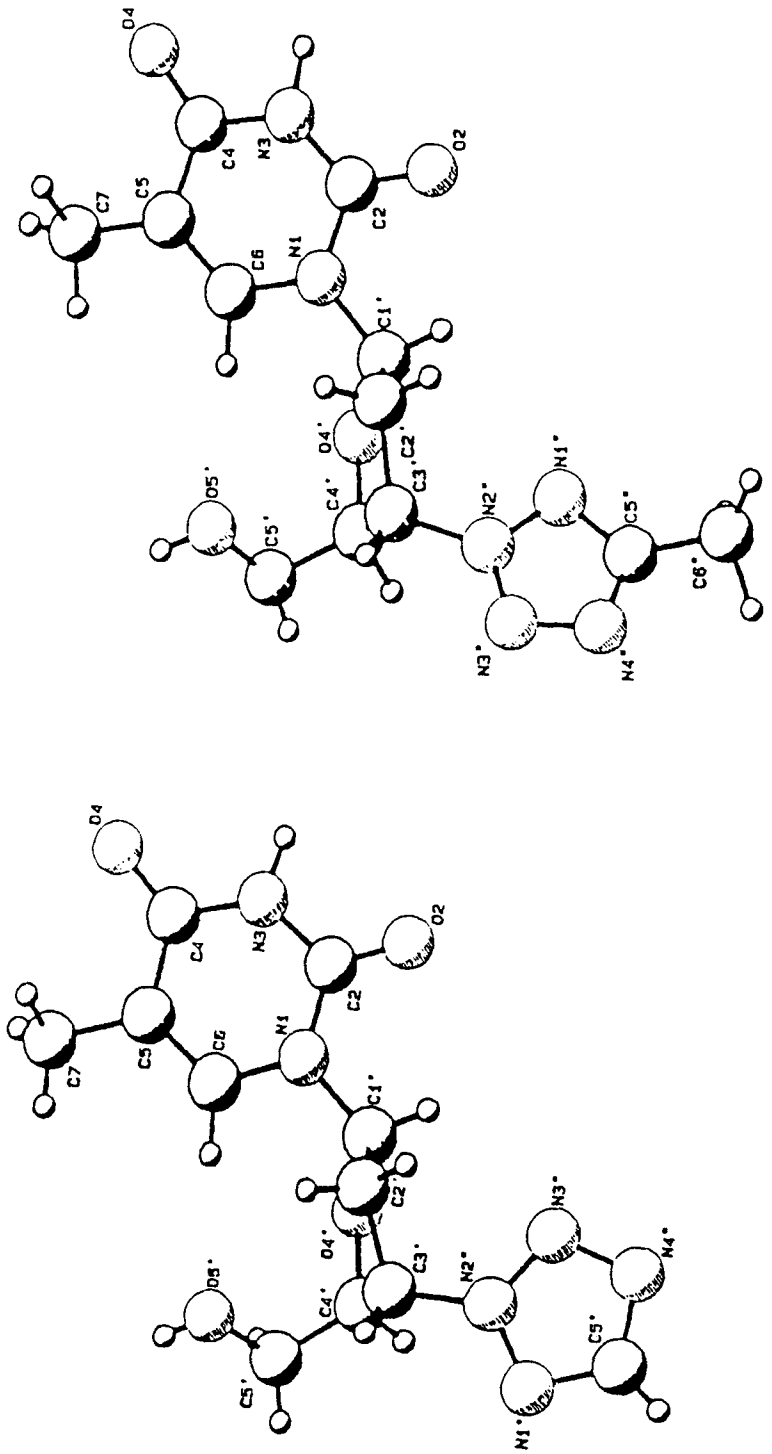


Figure 1. The structures of **Ia** and **Ib** molecules, and the accepted numbering of atoms

Table 4. Positional ($x10^4$) and thermal ($x10^3$) parameters for **Ia** and **Ib** structures

	Atom	x	y	z	Ueq ($Å^2$)*
Ia	O(2)	7126(10)	6501(4)	1050(2)	54(2)
	O(4)	658(10)	6253(4)	-103(2)	55(2)
	O(4')	7093(7)	2983(4)	1341(1)	39(1)
	O(5')	2669(8)	1117(4)	1296(2)	49(2)
	N(1)	4411(10)	4683(4)	1049(2)	33(2)
	N(3)	3911(10)	6317(5)	475(2)	38(2)
	N(1")	7476(19)	2000(7)	2829(2)	83(3)
	N(2")	6748(11)	2849(6)	2503(2)	41(2)
	N(3")	7964(13)	3976(7)	2545(2)	64(2)
	N(4")	9581(14)	3908(8)	2922(2)	71(3)
	C(2)	5286(12)	5886(6)	878(2)	39(2)
	C(4)	1841(11)	5705(6)	238(2)	35(2)
	C(5)	1180(10)	4415(6)	410(2)	33(2)
	C(6)	2465(12)	3963(6)	807(2)	31(2)
	C(7)	-930(13)	3660(6)	150(2)	45(2)
	C(1')	5681(11)	4113(6)	1479(2)	35(2)
	C(2')	3675(12)	3704(6)	1872(2)	42(2)
	C(3')	4999(11)	2503(6)	2092(2)	34(2)
	C(4')	6624(11)	1921(6)	1675(2)	37(2)
	C(5')	5379(13)	782(6)	1423(3)	46(2)
	C(5")	9227(19)	2700(10)	3088(3)	78(3)
	O(1)w	793(14)	-947(7)	749(2)	102(3)
Ib	O(2)	6159(21)	7916(6)	4651(7)	55(5)
	O(4)	-154(22)	8978(9)	3196(8)	60(5)
	O(4')	6804(16)	5461(6)	3776(5)	41(4)
	O(5')	2675(22)	4329(8)	3132(7)	56(4)
	N(1)	4091(23)	6794(8)	3973(7)	34(5)
	N(3)	2951(25)	8398(8)	3913(7)	40(5)
	N(1")	8053(22)	4499(9)	5443(8)	45(5)
	N(2")	6333(22)	3954(8)	5108(6)	34(4)
	N(3")	6117(28)	3065(9)	5363(7)	50(6)
	N(4")	7761(28)	3033(10)	5879(8)	55(6)
	C(2)	4519(29)	7737(11)	4204(9)	34(6)
	C(4)	1096(31)	8270(13)	3404(9)	40(7)
	C(5)	749(29)	7309(10)	3133(9)	34(5)
	C(6)	2197(29)	6616(11)	3449(8)	37(6)
	C(7)	-1193(37)	7128(9)	2606(9)	46(6)
	C(1')	5474(23)	5995(10)	4305(8)	33(5)
	C(2')	3560(27)	5284(10)	4651(8)	39(5)
	C(3')	4768(24)	4298(11)	4507(8)	42(6)
	C(4')	6445(33)	4440(9)	3852(8)	44(6)
	C(5')	5283(27)	4046(11)	3193(9)	39(6)
	C(5")	9017(31)	3893(12)	5918(9)	46(6)
	C(6")	10986(36)	4142(15)	6441(11)	71(8)

* Equivalent isotropic U defined as 1/3 of the trace of the orthogonalised U(i,j) tensor.

to the furanose ring is observed, the torsion angle χ(O4', C1', N1, C2) is equal to -113.0° for **Ia** and -125.8° for **Ib**. The pseudorotation phase angles P for the furanose rings are equal to 173.0° for **Ia** and 165.1° for **Ib**, the degree of pucker φ_m= 38.4° for **Ia** and 25.8° for **Ib**, i. e. in both compounds the furanose ring has C2'-*endo*-C3'-*exo* ($^2T_3$) conformation, with C2' and C3' atoms deviating from the planes of the C1', O4', and C4' atoms by 0.309 and 0.173 E for **Ia** and 0.366 and 0.037 E for **Ib**. The conformation of the molecules with respect to the exocyclic C4'-C5' bond is *gauche*$^+$ with torsion angles χ(O5', C5', C4', C3') = 50.3° (**Ia**) and 47.8° (**Ib**).

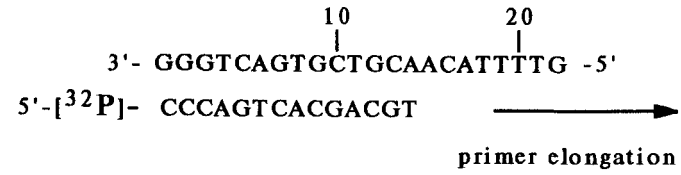
The structures of **Ia** and **Ib** molecules differ mainly by the orientation of their tetrazole rings. The tetrazole ring of **Ia** is rotated approximately by 180° around the C3'-N2" bond as compared with **Ib** (Fig. 1).

It should be noted that the conformation of **Ia** is similar to that of one of the crystallographically independent molecules of 3'-azido-3'-deoxythymidine [8] and markedly different from the conformation of 3'-amino- [9, 10] and 3'-methylamino-3'-deoxythymidine [11].

Tetrazolide nucleoside **Ia** was converted into the corresponding 5'-triphosphate **Va** by the reaction with *tris*-(1,2,4-triazolyl)phosphoryl oxide and then with pyrophosphate. We evaluated the substrate properties of triphosphate **Va** towards some reverse transcriptases and DNA polymerases using the system described in [12]. As the template-primer complex we used phage M13mp10 DNA and a complementary tetradecadeoxyribonucleotide labelled with [^{32}P] at the 5'-position (Scheme II).

As can be seen from Fig.2, incorporation of the nucleotide residue of **Va** into the 3'-position of the primer was observed only with AMV reverse transcriptase (series A). With 100 μM **Va** a new band corresponding to the pentadecanucleotide appears in the gel. The band in lane 2, where thymidilate residue is incorporated into the primer as the 15th nucleoside, served as a control. The presence of an extra band in lane 2 of series B is due to the error-prone properties of HIV reverse transcriptase.

Other enzymes (DNA polymerase α from human placenta, β from rat liver, KFr, and HIV reverse transcriptase) did not incorporate triphosphate **Va** into the DNA chain. This is evident from the absence of a pentadecanucleotide band on the lanes 3 and 4 in series C-E. Shorter oligonucleotides in the series E, bands 1, 3, and 4 result from hydrolysis of the primer by 3'→5' exonuclease activity of KFr. The corresponding fragments in the series C are due to contaminating exonuclease activity contained in the preparation of DNA polymerase α.



Scheme II

3'-Terminal region of phage M13mp10 DNA annealed with the tetradecanucleotide primer

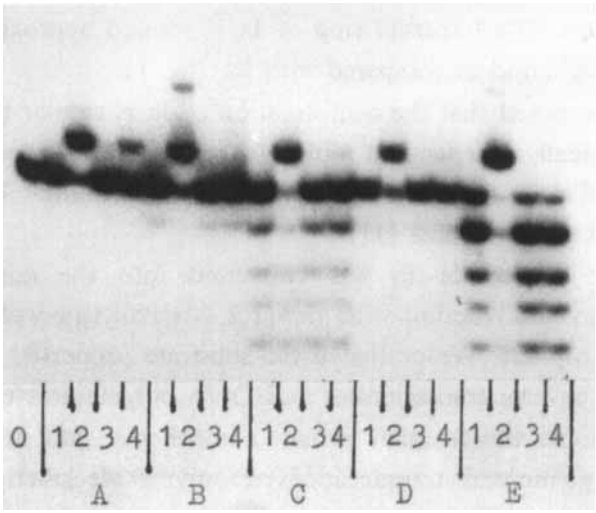


Fig. 2. Autoradiogram of the products of primer extension catalyzed by the AMV reverse transcriptase (A), HIV reverse transcriptase (B), DNA polymerases α (C), β (D), and KFr (E). 0 - template-primer complex; 1 - as 0 + enzyme; 2 - as 1 + 10 μM dTTP; 3 - as 1 + 10 μM **Va**; 4 - as 1 + 100 μM **Va**.

Since incorporation of **Va** into DNA (lane 4, series A) was observed only at a concentration of 100 μM and only to a slight degree [no incorporation of **Va** is observed at 10 μM, (lane 3, series A)] we conclude that the affinity of **Va** to the DNA synthesizing complex is quite low. It should be pointed out that in the same conditions 10 μM dTTP entirely converts the primer into the pentadecanucleotide (lane 2, series A).

Synthesis of 5"-substituted 3'-N-(tetrazole-2"-yl)-3'-deoxythymidine by interaction of 5'-protected 2,3'-O-anhydrothymidine with the salts of the corresponding tetrazoles made it possible to obtain a large group of stable 3'-modified nucleosides. The crystal conformation of at least two such molecules is very close to that of the crystalline form of 3'-azido-3'-deoxythymidine [8]. However, unlike 3'-azido-3'-deoxythymidine 5'-triphosphate, **Va** manifests poor substrate properties only towards AMV reverse transcriptase in the series of enzymes under testing. Evidently, only data on the crystal conformation of flexible nucleosides are not sufficient to predict substrate properties of their 5'-triphosphates towards DNA polymerases.

Experimental section

Chemistry. ^1H NMR spectra (200 MHz) and ^{13}C NMR spectra (50.3 MHz, proton decoupling, internal standard DMSO-d_6) were registered on a Bruker AC-200 spectrometer. Mass spectrometry (electron pulse method) was carried out on an MX 1308 spectrometer (70 eV). TLC was performed on Silufol UV-254 (Kavalier) plates in ethyl acetate and DEAE cellulose DE-32 (Whatman). Tetrazole and 5'-substituted tetrazoles were obtained by the methods described in [6], 5'-O-benzoyl-2,3'-anhydrothymidine was prepared according to [13]. The results of elemental analyses for compounds **Ia-d** and **IVa-d** for C, H, and N are consistent with calculated values.

Crystals of **Ia** and **Ib** for X-ray analysis were grown from saturated solutions of these compounds in a water-ethanol mixture by slow evaporation of the solvent at room temperature. Their space group is $P2_12_12_1$, the cell dimensions are: a= 4.952(1) Å [5.067(4) Å]; b= 10.212(2) Å [13.923(5) Å]; c= 27.506(5) Å [19.068(7) Å]; V= 1391.0 Å^3 [1345.2 Å^3]. z= 4·(**Ia** molecule·H_2O) [4·**Ib** molecule] (data in square parentheses belong to **Ib** crystal). X-ray data collection was performed on a CAD-4 diffractometer (MoK_α radiation with a β-filter, the θ/2θ scanning technique). The structures were determined by direct methods and refined by the full-matrix least square technique to R=0.037 (**Ia**) and 0.059 (**Ib**) using the anisotropic approximation for nonhydrogen atoms.

We used DNA polymerase α from human placenta [14], DNA polymerase β from rat liver [15], and HIV reverse transcriptase [16]. Other enzyme preparations were commercial. The primer tetradecanucleotide was labelled at the 5'-position, using [γ-^{32}P]ATP (specific activity 1500 Ci/mmol, Radioizotop) and phage T4 polynucleotide kinase as described in [17]. Phage M13mp10 DNA was hybridized with the [5'-^{32}P]-labelled primer in the

following buffers: 10 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 1 mM dithiotreitol (for KFr); 10 mM Tris-HCl (pH 8.2), 5 mM $MgCl_2$, 40 mM KCl, 1 mM dithiotreitol (for reverse transcriptases); 10 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$, 0.4 mM dithiotreitol (for DNA polymerase α); the same as for DNA polymerase α, but with pH 8.5 (for DNA polymerase β).

5'-O-Benzoyl-3'-(tetrazole-2"-yl)-3'-deoxythymidine (IVa). A mixture 5'-O-benzoyl-2,3'-anhydrothymidine **II** (20 g, 0.061 mol) with tetrazole (5.04 g, 0.072 mol) and triethylamine (10.08 ml, 0.072 mol) in DMF (120 ml) and 1,4-dioxane (20 ml) was heated for 40 h at 100°C, then cooled, filtered, and the solvent was evaporated. The obtained oily liquid (22.9 g) was dissolved in 400 ml of chloroform, washed with water, dried with $MgSO_4$, diluted with chloroform to 1 l, and filtered through the 5 cm layer of silica gel L 40/100 (Chemapol). The solvent was evaporated, and the resulting oily liquid was recrystallized from ethanol. Yield 7.2 g.

Compounds **IVb-d** were obtained by a similar technique (Tables 1-3).

3'-(Tetrazole-2"-yl)-3'-deoxythymidine (Ia). A mixture of 5'-O-benzoyl-3'-(tetrazole-2"-yl)-3'-deoxythymidine **IVa**) (9.15 g, 0.024 mol) in ethanol (65 ml) with 33% aqueous dimethylamine (26 ml) was stirred for 10 min at 20-25°C and then heated for 40 min at 60°C (TLC control). The solvent was evaporated, the residue was coevaporated with water (5 x 400 ml), recrystallized from water in the presence of activated charcoal. Yield 2.5 g.

Compounds **Ib-d** were obtained by a similar method (Tables 1-3).

3'-(Tetrazole-2"-yl)-3'-deoxythymidine 5'-triphosphate (Va). 1,2,4-Triazole (22 mg, 0.3 mmol) was dissolved in acetonitrile (0.5 ml); then triethylamine (44 μl, 0.3 mmol), and phosphorus oxychloride (10 μl, 0.1 mmol) were added and after stirring for 40 min at 20°C the precipitate was removed by centrifugation, and **Ia** (21 mg, 0.07 mmol) was added. The reaction mixture was stirred for 1 h at 20°C, 0.5 M bis-(tri-n-butylammonium)pyrophosphate in DMF (1 ml, 0.5 mmol) was added, and the mixture was kept for 1 h at 20°C. The reaction mixture was diluted with water to 50 ml, loaded onto a DE-32 cellulose column (3 x 10 cm, HCO_3^-). The compounds were eluted with a linear gradient of 0 → 0.3 M NH_4HCO_3, pH 7.5 (total volume 1 l). UV-absorbing fractions were evaporated, the residue was reevaporated with water:ethanol (1:1 v/v). Yield 11 mg (29%). ^1H NMR spectrum (D_2O; δ, ppm; *J*, Hz): 8.37s (1H, tetrazole); 7.78q (1H, H-6); 6.52m (1H, *J* 7, H-1'); 5.95m (1H, H-3'); 4.53m (1H, H-4'); 4.31m (2H, H-5'); 2.88m (2H, H-2'), 1.94d (3H, *J* 1, CH_3). ^{31}P NMR spectrum (D_2O; δ, ppm): -8.3d (P_γ), -10.8d (P_α), -22.1t (P_β); $J_{P_\alpha,P_\beta}=J_{P_\beta,P_\gamma}=22$ Hz.

Primer extension assays were carried out as described in [12]. Incorporation of the nucleotide residue into the 3'-terminus of the [5'-^{32}P]-primer was performed in the incubation mixture (6 μl), containing 0.01 μM template-primer complex, 10 μM dNTP or 10 μM (100 μM) **Va** , the enzyme (3 activity units of reverse transcriptases, 1 activity unit of DNA polymerases α and β, 0.4 activity unit of KFr), and the corresponding buffer. The reaction was carried out for 20 min at 37°C (10 min at 20°C for KFr) and terminated by the addition of 3 μl of formamide, containing EDTA and dyes. The reaction products were separated by 20% PAGE. Autoradiographic assays were performed using XRP-5 X-ray films (Kodak).

Acknowledgments.

This work was supported by the Russian Fund of Fundamental Research (grants № 93-04-7959 and № 93-04-20542), and the Program "National Priorities in Medicine and Public Health: AIDS (grant Sp. 306). Authors are grateful to Drs. V.A. Gindin and V.V. Takchistov for their assistance in measuring and interpretating NMR and mass-spectra.

References

1. Mitsuya, H.; Yarchoan, R.; Broder, S. *Science*, 1990, **249**, 1553-1544.
2. De Clercq, E. *Med. Res. Rev.*, 1992, **13**, 229-258.
3. Krayevsky, A.A. *Molec. Biology (Russian)*, 1992, **26**, 725-744
4. Wigerinck, P.; Van Aerschot, A.; Janssen, G.; Claes, P.; Balzarini, J.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.*, 1990, **33**, 868-873.
5. Habich, D. *Synthesis,* **1992**, 358-360.
6. Koldobskii, G.I.; Ostrovskii, V.A.; Poplavskii, V.S. *Chem. Heterocyclic Comp.* (Russian), **1981**, 1299-1326.
7. Koren, A.O.; Gaponik, P.N.; Ostrovskii, V.A. *Int. J. Chem. Kinetics,* 1993, **25**, 1043-1051.
8. Gurskaya, G.V.; Tsapkina, E.N.; Scaptsova, N.V.; Krayevsky, A.A.; Lindeman, S.V.; Struchkov, Ju.T. *Dokl. Acad. Nauk SSSR*, 1986, **291**, 854-859.
9. Gurskaya, G.V.; Tsapkina, E.N.; Lindeman, S.V.; Struchkov, Ju. T.; Krayevsky, A.A. *Dokl. Acad. Nauk SSSR*, 1988, **303**, 1378-1382.
10. Kovacs, T.; Parkanyi, L.; Pelczer, I.; Cervantes-Lee, F.; Pannell, K.H.; Torrence, P.F. *J. Med. Chem.,* 1991, **34**, 2595-2600.

11. Bochkarev, A.V.; Jasko, M.V.; Zdanov, A.S.; Fedorov, I.I.; Gurskaya, G.V. *Bioorg. Chem. (Russian)*, 1992, **18**, 996-1001.
12. Victorova, L.S.; Dyatkina, N.B.; Mozzherin, D.Ju., Atrazhev, A.M.; Krayevsky, A.A.; Kukhanova, M.K. *Nucl. Acids. Res.*, 1993, **20**, 783-789.
13. Zaitseva, V.E.; Dyatkina, N.B.; Krayevsky, A.A.; Skaptsova, N.V.; Turina, O.V.; Gnuchev, N.V.; Gottikh, B.P.; Azhayev, A.V. *Bioorg. Chem. (Russian)*, 1984, **10**, 670-680.
14. Mozzherin, D.Yu.; Atrazhev, A.M.; Kukhanova, M.K. *Molec. Biology (Russian)*, 1992, **26**, 999-1010.
15. Atrazhev, A.M.; Kukhanova, M.K. *Bioorg. Chem. (Russian)*, 1985, **11**, 1627-1635.
16. Rozovskaya, T.A.; Belogurov, A.A.; Lukin, M.A.; Chernov, D.N.; Kukhanova, M.K.; Beabealashvilli, R.Sh. *Molec. Biol. (Russian)*, 1993, **27**, 618-630.
17. Sambrook, J.; Fritsch, E.F.; Maniatis, T. Molecular Cloning. A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press. N.-Y. 1989.

Received June 20, 1994
Accepted January 10, 1995