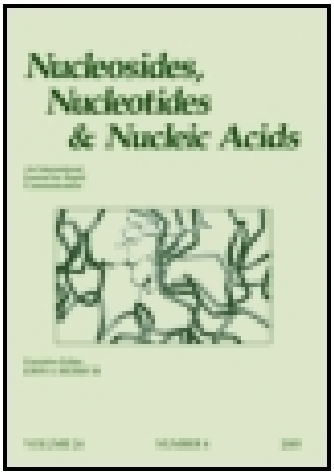

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Synthesis, Molecular and Crystal Structure of 3′-N-Alkylamino-3′-deoxythymidines and Some Biochemical Properties of Their Phosphorous Esters

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SYNTHESIS, MOLECULAR AND CRYSTAL STRUCTURE OF 3'-N-ALKYLAMINO-3'-DEOXYTHYMIDINES AND SOME BIOCHEMICAL PROPERTIES OF THEIR PHOSPHOROUS ESTERS

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Abstract: Two approaches for the synthesis of 3'-N-alkylated 3'-amino-3'-deoxythymidines were developed, including both the reaction of 3'-amino-3'-deoxythymidine with aldehydes followed by the sodium borohydride reduction and preparation of the triphenylphosphinimine derivative and subsequent reaction with alkyl iodide. 5'-Triphosphates of the synthesized compounds were shown to be terminating substrates for human immunodeficiency virus and avian myeloblastosis virus reverse transcriptases as well as for DNA polymerase β from rat liver. At the same time these compounds did not demonstrate the properties of a terminating substrate in DNA synthesis catalyzed by human DNA polymerases α, ε, and I from *E. coli*.

Abbreviations used: dT(3'N_3), dT(3'NH_2), dT(3'NHMe), and dT(3'NHEt) - 3'-azido-, 3'-amino-, 3'-methylamino-, and 3'-ethylamino-3'-deoxythymidines, respectively; dTTP(3'N_3), dTTP(3'NH_2), dTTP(3'NHMe), and dTTP(3'NHEt) - 5'-triphosphates of dT(3'N_3), dT(3'NH_2), dT(3'NHMe), and dT(3'NHEt), correspondingly; RT - reverse transcriptases of human

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immunodeficiency virus type 1 (HIV) and avian myeloblastosis virus (AMV); KFr - *E. coli* DNA polymerase I, Klenow fragment.

Introduction

Among 2'-deoxynucleoside analogs modified at the 3'-position, dT(3'NH_2) [1], attracts definite attention. Its 5'-triphosphate, dTTP(3'NH_2) shows the property of a potent terminating substrate for many DNA polymerases including the replicative enzymes α and ε [2, 3]. As a precursor of the efficient DNA polymerase inhibitor, dT(3'NH_2) is still of no use in clinical practice because of its strong toxicity [4-6]. But the search of new active and selective inhibitors of HIV reproduction among derivatives of dT(3'NH_2) seems to be promising. Retroviral RTs are known to be less specific towards nucleotide substrates than DNA polymerases of high organized biological systems [7, 8]. This prompted us to introduce additional modifications into the molecule of dT(3'NH_2). Such attempts have already been made by at least two groups of researchers but the obtained 3'-N-acylated dT(3'NH_2) derivatives [9-11] did not show significant antiviral activity. Based on these data we suggested that the following rules should be kept in mind to retain the inhibiting properties dTTP(3'NH_2) derivatives towards HIV RT: (i) retention of the basic properties of the nitrogen atom at C3'; and (ii) introduction of additional substitutents to the 3' nitrogen of the molecule. These considerations prompted us to synthesize 3'-N-alkylated derivatives of dT(3'NH_2) and examine their ability to inhibit the reproduction of retroviruses. The 5'-triphosphates of these compounds were used to evaluate the substrate specificity of several DNA polymerases and reverse transcriptases. Our preliminary data on the synthesis of dT(3'NHMe) have been reported in [12].

Results and Discussion

Synthesis. Our first attempts to prepare 3'-N-mono- and 3'-N-dialkylated derivatives of 3'-amino-3'-deoxythymidine [dT(3'NH_2)] starting from 1-(3-O-trifluoromethanesulfonyl-5-O-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine in reaction with alkylamines afforded exclusively to 3'-deoxy-2',3'-didehydrothymidine formation similarly to the reaction of the corresponding methanesulfonyl compound [13]. The standard method of alkylation of the dT(3'NH_2) amino group by alkyl halides in the presence of bases resulted in the alkylation of thymine N-3. During the reaction of dT(3'NH_2) with MeI or EtI

in ethanol in the presence of K_2CO_3 3-N-alkyl- (1 or 2) as well as 3,3'-N-dialkyl derivative 3 were identified (Scheme I).

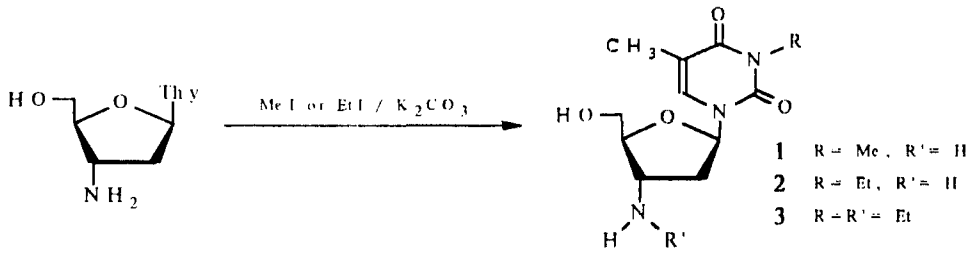
The involvement of thymine N-3 atom in the reaction with alkyl halides was corroborated by ^1H NMR data. In the spectrum of compound 1 the chemical shift of 3-N-Me group is 3.29, for compound 2 - chemical shift of 3-N-methylene group of Et is 3.95 ppm. The resonance of 3-N-CH_2 appeared at 3.92 ppm and at 2.63 ppm for 3'-N-CH_2 in compound 3 (complete data are not given). The values of chemical shifts for the protons of the substituent at alkyl α-carbon atom were in good correlation with those for a range of 3-N-alkyl pyrimidines reported [14]. These results made it possible to develop other synthetical approaches to obtaining 3'-N-alkylaminothymidines. For this purpose dT(3'N_3) was converted into title dT(3'NHMe) and dT(3'NHEt) 5 and 6 *via* the intermediate step of triphenylphosphine derivative formation by treating with alkyl iodides in dioxane (Scheme II). But the extremely low yields of target compounds in these reactions prompted us to develop another synthetic approach which allowed access to 3'-N-dimethylamino derivative 4. It should be mentioned that similar reaction conditions were used earlier to obtain 3'-N-alkylated nucleoside derivatives containing hydroxyl at C2' [15]. The dT(3'NH_2) was treated with paraformaldehyde solution in methanol with subsequent reduction with sodium borohydride to obtain the mixture of desirable 5 (72%) and 4 (8%). In the case of acetaldehyde 3'-N-dialkylamino derivative was not detected.

Separation of compounds 4 and 5 was performed by LiChroprep RP-18 chromatography (elution with 0.05 M NH_4HCO_3).

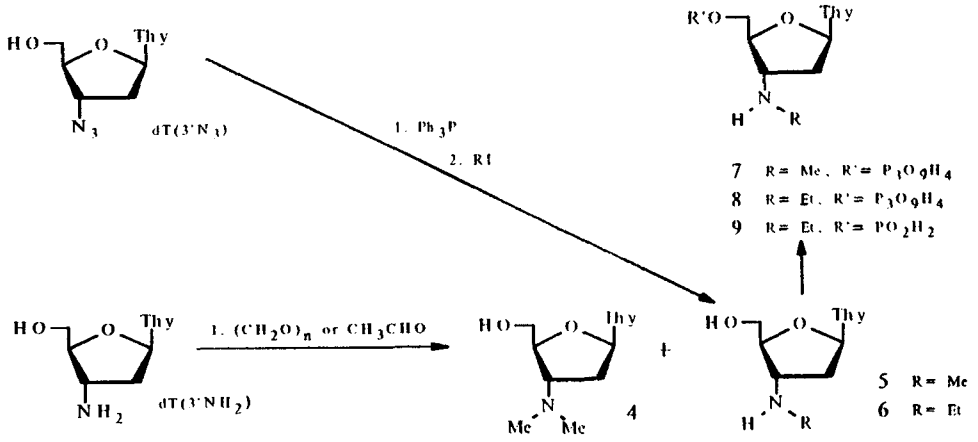
5'-Hydrogenphosphonates of modified nucleosides appeared to be in some cases less toxic agents inhibiting viral reproduction in cell cultures [16]. Therefore we transformed 3'-N-ethylamino-3'-deoxythymidine 6 into the corresponding 5'-hydrogenphosphonate 9 using the routine procedure reported in [17].

We also converted 3'-N-methylamino- and 3'-N-ethylamino-3'-deoxythymidines 5 and 6 into corresponding 5'-triphosphates 7 and 8 by the reaction with $POCl_3$ in triethylphosphate and subsequent treating with bis-(tri-n-butylammonium) pyrophosphate [18].

The structure of the obtained compounds was confirmed by ^1H, ^{13}C, and ^{31}P NMR, UV, mass-spectroscopy as well as by X-ray analysis for compound 5. UV spectra of the title compounds were the same as for dT(3'NH_2) (data are not presented). All sugar protons of 3'-N-alkylamino-3'-deoxythymidines 4-9



Scheme I



Scheme II

resonated in approximately the same areas as the corresponding protons of dT(3'NH_2) (Table I). The additional proton signals for the alkyl groups in N3'-position were observed in 2.58-2.16 ppm area. An essential spectral difference of 3'-N-mono alkylated derivatives in comparison with dT(3'NH_2) was in the visual picture of signal H1' as pseudotriplet whereas in dT(3'NH_2) this signal was a clear doublet of doublets (Tables I and II). Chemical shift values in ^{13}C NMR spectra are represented in Table III.

Structures in crystal and solution. Conformational comparison dT(3'NHMe) (5) with dT(3'NH_2) was performed by X-ray analysis as well as by pseudorotational analysis of all the experimental endocyclic $^3J_{H,H}$ coupling constants (taken from the experimental spectra in D_2O solution) for both of these nucleosides using PSEUROT program [19, 20].

Table I. Chemical Shifts (δ, ppm) in ^{1}H NMR Spectra

com-pound	H-6	H-1'	H-4'	H-5'	H-5''	H-3'	H-2'	H-2''	5-Me	other
dT(3'NH_2)	7.68 q	6.22 dd	3.86 m	3.91 dd	3.79 dd	3.6 m	2.4 m	2.27 m	1.88 d	
4	7.54 q	6.07 pt	4.04 m	3.76 m	3.62 dd	3.15 dd	2.43 m	2.20 m	1.77 d	(Me_2-N) 2.16s
5	7.61 q	6.18 pt	3.92 td	3.82 dd	3.70 dd	3.34 td	2.36 m	2.27 m	1.83 d	(Me-N) 2.32s
6	7.58 q	6.15 pt	3.84 m	3.81 dd	3.69 dd	3.38 m	2.34 m	2.24 m	1.76 d	(CH_2-N) 2.58q (CH_3-Et) 1.02t
7	7.63 q	6.23 pt	3.91 -- 4.5 m				2.45 m		1.93 d	(Me-N) 2.39s
8	7.64 q	6.26 pt	4.00 -- 4.52 m				2.63 m		1.94 d	(CH_2-N) 3.09q (CH_3-Et) 1.34t
9	7.61 q	6.26 pt	3.95 -- 4.13 m				2.59 m		1.82 d	(CH_2-N) 3.08q (CH_3-Et) 1.22t (H-P) 6.73d ($J_{H,P}$641.8)

Table II. Spin-Spin Coupling Constants (*J*, Hz) in ^{1}H NMR Spectra

compound	1',2'	1',2''	2',2''	2',3'	2'',3'	3',4'	4',5'	4',5''	5',5''
dT(3'NH_2)	4.5	7.3	14.1	7.8	7.4	7.1	2.9	4.8	12.7
4	6.6	6.6	14.5	7.2	8.5	5.8	2.6	5.5	12.4
5	5.8	6.8	14.2	7.6	6.0	6.0	3.2	4.9	12.6
6	6.0	6.5	14.0	7.7	7.0	5.5	3.0	4.8	12.5

Table III. Chemical Shifts (δ, ppm) in ^{13}C NMR Spectra

com-pound	C4	C2	C6	C5	C1'	C4'	C5'	C3'	C2'	Me-C5	other
4	170.3	154.9	139.9	113.8	88.1	84.9	66.7	65.1	43.7	14.2	(Me_2-N) 34.9
5	169.7	154.7	139.8	113.6	87.6	87.2	64.1	60.9	38.9	14.1	(Me-N) 35.4
6	167.8	152.8	138.0	111.8	85.8	85.6	62.3	57.3	37.4	14.1	(CH_2-N) 42.2, (CH_3-Et) 12.2

The content of the predominant N pseudorotamer in the equilibrium of two states N (3'-*endo*-2'-*exo*) and S (3'-*exo*-2'-*endo*) appeared to be slightly different for dT(3'NH_2) (75%) and 5 (58%). However, these calculations for the phase angle (P) and puckering amplitude (Ψ_m (N)) of the N sugar of **5** gave the values of 19° and 30°, respectively. These parameters were approximately the same for the parent dT(3'NH_2) (18° and 30°, respectively). For minor S pseudorotamers, the values of P for both nucleosides were also identical (approximately 160°). Exocyclic coupling constants $J_{4',5'}$ and $J_{4',5''}$ gave the distribution of γ^{g+}, γ^{t} and γ^{g-} relative to the staggered exocyclic C4'-C5' bond. Thus, the relative content of γ^{g+} rotamer in dT(3'NH_2) and **5** was 58% and 55%, γ^{t} - 36% in both cases and γ^{g-} - 6% and 9%.

Fig. 1 shows the conformation of the dT(3'NHMe) molecule obtained from X-ray data and the accepted numbering of the atoms. The bond lengths and bond angles in **5** correlate well with the same values for the nearest analogs: natural thymidine [21], dT(3'N_3) [22], and dT(3'NH_2)·HCl [23]. As for the majority of pyrimidine nucleosides, the glycosidic torsion angle (χ = -106.7°) corresponds to the *anti*-conformation. For dT(3'NH_2)·HCl, the value of χ= -167° is significantly different. A χ value, much closer to **5**, has been found in A-molecule dT(3'N_3) (χ=-125°). In this case the best coincidence of bond lengths and angles was observed.

The replacement of OH-group at C3' in thymidine by Me-NH-group results in the change of conformation of deoxyribose moiety. The sugar

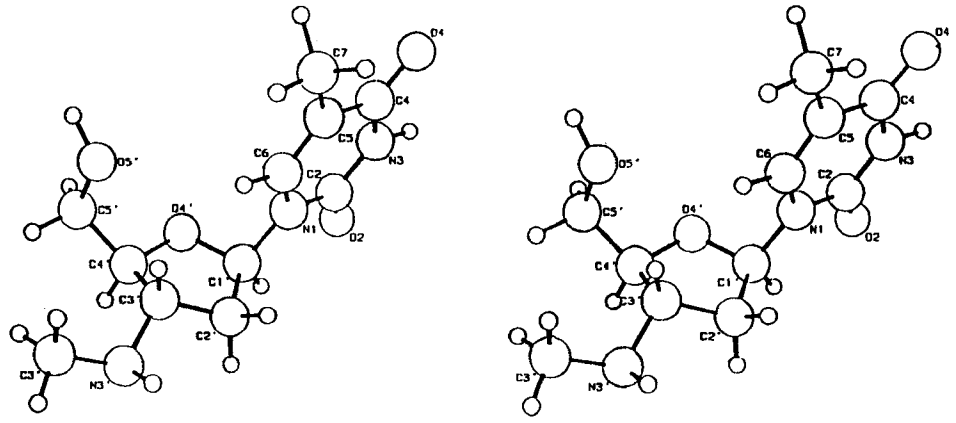
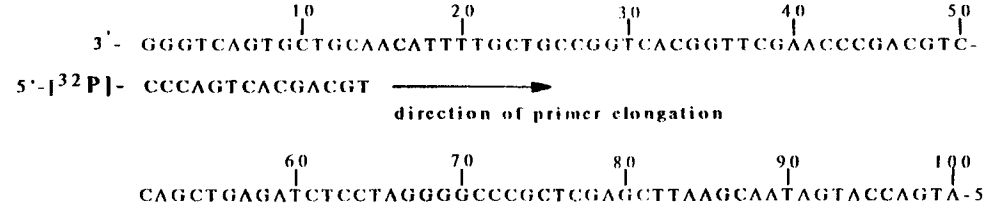


Figure 1. Stereo diagram showing the molecular conformation and the numbering of atoms of 5.

conformation in dT(3'NHMe) is C3'-*endo*-C4'-*exo* ($^3T_4$), with phase angle P=28.8° (N-population) and puckering amplitude Ψ_m=31.5°. C3' and C4' atoms are deviated by 0.36 Å and 0.14 Å, respectively, from the least-squares plane through atoms O4', C1' and C2'. The molecule of dT(3'NH_2)·HCl possesses phase angle value P=3.8° and C3'-*endo*-C2'-*exo* sugar conformation, also belonging to N-population. In this case atoms C3' and C2' are displaced from the plane of O4', C1' and C4' by 0.36 Å and 0.24 Å. Thymidine has P=187.5° and C3'-*exo*-C2'-*endo* conformation and dT(3'N_3) - P=173°, molecule A; P=212°, molecule B (S-population) that greatly differs from 5. However, 3'-deoxythymidine, which exhibits some anti-HIV activity [24], is characterized by the sugar conformation (P=12.9°) close to that in compound 5.

The conformation about the exocyclic C4'-C5' bond in crystal appeared to be *gauche*$^+$ with γ (C3'-C4'-C5'-O5')=45.8°. Similar conformation has been observed in dT(3'NH_2)·HCl where γ =57° and in dT(3'N_3) molecule A (γ=50.8°). For thymidine as well as for dT(3'N_3) molecule B are characteristic *trans*- state with γ=172.8° and 173.5°, respectively.

Taken together, the data of X-ray and pseudorotational analysis show that the molecules of 3'-amino-3'-deoxythymidine and 3'-methylamino-3'-deoxythymidine 5 have similar conformations in crystal as well as in aqueous solution: (i) about glycosidic bond - *anti*; (ii) with respect to exocyclic C4'-C5'-bond *gauche*$^+$; (iii) furanose ring conformation belongs to the N-population.



Scheme III. Primer-Template Complex

Enzymology. The substrate properties of the nucleoside 5'-triphosphates 7 and 8 were evaluated in cell free systems with various DNA polymerases. The 5'-[^{32}P]-primer-template complex (Scheme III), dNTP, dTTP(3'NHMe), or dTTP(3'NHEt) at appropriate concentrations were present in the reaction mixtures. The reaction was performed under the optimal conditions for each enzyme. The reaction products were separated by PAGE.

dTTP(3'NHMe) (7) and dTTP(3'NHEt) (8) did not terminate the elongation of DNA chain catalyzed by DNA polymerases α and ε from human placenta and KFr at 200-fold molar excess of the compounds over dTTP. At the same time the elongation of the DNA chain catalyzed by AMV RT, HIV RT and DNA polymerase β from rat liver is terminated by both of the compounds with different efficiencies. In these experiments RTs incorporated 7 and 8 into DNA at least 10-fold more efficiently than DNA polymerase β from rat liver. Fig. 2 shows the DNA termination pattern obtained for 5'-triphosphates 7 and 8 with HIV and AMV RTs. dTTP(3'N_3) was used in control assays (track 1). The termination patterns obtained for a 5-fold excess of dTTP(3'N_3) over dTTP was similar to that for a 10-fold excess of 7 and 8.

Table IV shows the molar ratios of 7 and 8 concentrations to that of dTTP, at which DNA synthesis catalyzed by different DNA polymerases is inhibited by 50%. It is clear from the Table that 7 and 8, unlike dTTP(3'NH_2), do not inhibit KFr, DNA polymerases α and ε. Thus, introduction of the alkyl residue into 3'-amino group reduces the efficiency of inhibition. This fact indicates that bulky lipophylic groups (methylamino or ethylamino groups instead of amino group) decrease the affinity of modified dNTP for these DNA polymerases.

dT(3'NHMe) (5), dT(3'NHEt) (6), and dT(3'NHEt) 5'-hydrogenphosphonate 9 showed no suppression of HIV-1 reproduction in MT4

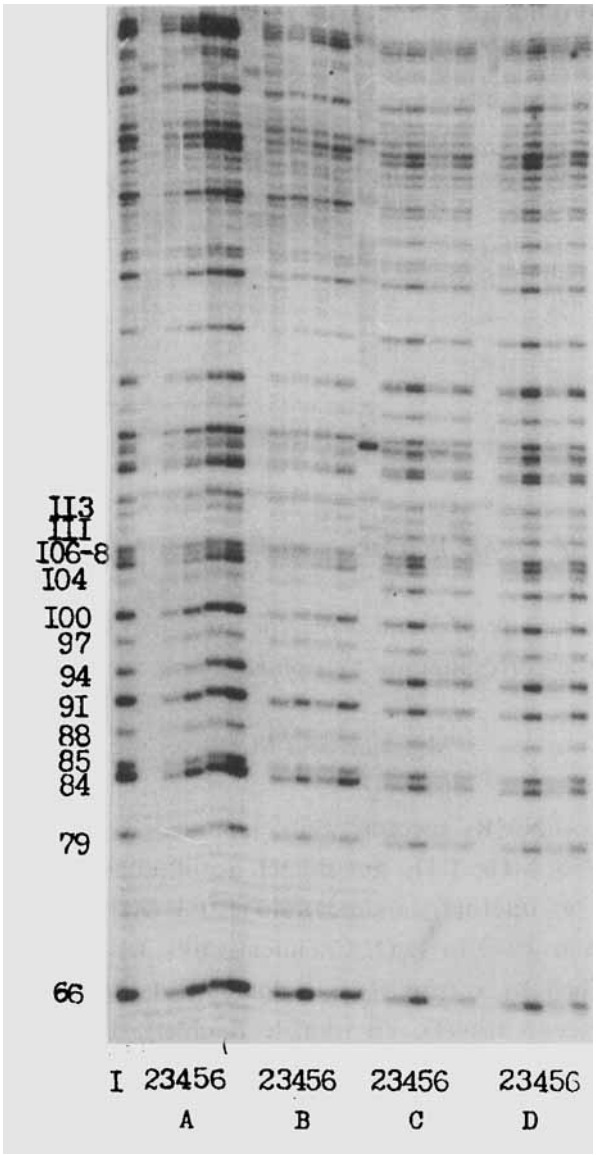


Figure 2. Separation by PAGE of the products of 5′-[^{32}P]-primer elongation catalyzed by HIV-1 RT (series A, B) and AMV RT (series C, D) in the presence of compounds *1* – dTTP(3′N_3) 12 μM; *2* – control assay without modified nucleotides; *3-4* – dTTP(3′NHMe), 18 μM and 60 μM for HIV RT; 60 μM and 180 μM for AMV RT, respectively; *5-6* – dTTP(3′NHEt) 18 μM and 60 μM for HIV RT; 60 μM and 180 μM for AMV RT, respectively. The assays in series *B* and *D* were chased with the mixture of 400 μM dNTPs.

Table IV. Molar ratios of 7 and 8 concentrations to that of dTTP at which DNA synthesis is inhibited by 50%

DNA polymerase	Inhibitor / dTTP molar ratio			
	dTTP(3'N_3)	dTTP(3'NH_2)	dTTP(3'NHMe)	dTTP(3'NHEt)
RT HIV	5	12	30	30
RT AMV	3	12	180	300
DNA pol. β	300	6	900	1200
DNA pol. α	>1200	120	>1200	>1200
KFr	>1200	600	>1200	>1200

cell culture (data of Drs J. Balzarini and E. De Clercq to whom authors are grateful).

The lack of activity of **5** and **6** may be explained by the fact that they are not converted to the corresponding 5'-triphosphates.

Materials and Methods

Chemistry. NMR spectra were recorded on a Bruker WM-250 spectrometer at 250 MHz (^{1}H, *tert*-BuOH as internal standard), 62.8 MHz (^{13}C, 1,4-dioxane as internal standard) and 101.3 MHz (^{31}P, 85% H_3PO_4 as external standard) at 25°C in D_2O. Chemical shifts are given in ppm (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), pt (pseudo triplet), dd (double doublet). FAB mass-spectra were recorded on a Kratos MS 50TC mass spectrometer. Samples were mixed with glycerol directly in the probe tip and xenon was used for the fast atom gun at 8 Kev. DEAE-Cellulose DE-32 were from Whatman, LiChroprep RP-18 (25-40 μm) and DC-Alufolien Kieselgel 60 F254 plates from Merck, Dowex 50 WX 8 from Serva. For TLC next systems were used (v/v): A - dioxane : NH_3 (25%), 9 : 1, B - dioxane : NH_3 (25%), 9 : 2. The X-ray analysis of dT(3'NHMe) was carried out on a CAD-4 diffractometer; the crystal was grown from D_2O.

3'-N-Methylamino-3'-deoxythymidine (5) and 3'-N-dimethylamino-3'-deoxythymidine (4) Method A. Triphenylphosphine (73 mg, 0.28 mmol) was added to a solution of dT(3'N_3) (62 mg, 0.23 mmol) in dioxane (20 ml). Reaction mixture was kept for 24 h at room temperature and, after addition of

MeI (1 ml), was boiled for 40 min, evaporated to dryness, the residue was dissolved in water (20 ml), filtered and evaporated. The residue in water (1 ml) was loaded onto the column (12 x 1.5 cm) with LiChroprep RP-18 and eluted with a linear gradient of MeOH in water (0 -› 4%, total volume 0,3 l) to give 5 (9 mg, 15%), R_f 0.24 (A). FAB mass spectrum: m/z 256 (MH^+).

Method B. Paraform (1 g) in dry ethanol (20 ml) was added to a solution of dT(3'NH_2) (300 mg, 1.24 mmol), the solution was stirred 48 h at room temperature and then sodium borohydride (200 mg, 5.3 mmol) was added by 3 equal portions with 1 h intervals. After 3 h the solution was treated with water (20 ml), evaporated, solved in water (200 ml), placed to the column (2 x 8 cm) with Dowex 50 (H^+), washed with water (200 ml) and then eluted with 1% aqueous ammonia. The target fractions with UV absorbtion were evaporated, solved in water (5 ml), placed onto the column with LiChroprep RP-18 and eluted MeCN/0.05 M NH_4HCO_3 (0 → 6% MeCN, total volume 0.5 l) to give 5 (183 mg, 72%) and **4** (25 mg, 8%), R_f 0.32 (A), FAB mass spectrum: m/z 127 (BH^+), 270 (MH^+).

3'-N-Ethylamino-3'-deoxythymidine (6). Method A as described for synthesis of compounds **5** with using of EtI allowed to get **6** with 25% yield. (A: R_f 0.33). FAB mass spectrum: m/z 127 (BH^+),144 (M^+-B), 270 (MH^+). Method B as described for synthesis of compounds **4** and **5** with using of acetaldehyde provided **6** with 57% yield.

3'-N-Methylamino-3'-deoxythymidine 5'-triphosphate (7) (ammonium salt). $POCl_3$ (11 µl, 0.12 mmol) was added to 5 (10 mg, 0.04 mmol) in triethyl phosphate (0.4 ml) at 0°C, the solution was stirred for 48 h at 0°C and then a 0.5 M bis-(tri-n-butylammonium) pyrophosphate in DMF (1.0 ml) and tributylamine (86 µl) at 0°C were added. The reaction mixture was stirred for 30 min at 20°C and then neutralized with 1M $Et_3NH_2CO_3$ to adjust the pH to 7.5, evaporated at 20°C, and reevaporated with aqueous EtOH. The residue in water (150 ml) was applied onto a DEAE cellulose column (HCO_3^-). The substances were eluted with a linear gradient of NH_4HCO_3, pH 7.5 (0 -› 0.4 M, 2 l), 7 was eluted with 0.25 M buffer. The fractions containing 7 were evaporated at 20°C and reevaporated with 5% EtOH (5 x 10 ml). After freeze-drying, 7 (6 mg, 26%) was obtained. ^{31}P NMR (D_2O): δ -8.3d (P_γ), -10.8d (P_α), -22.1t (P_β); $J_{P\alpha,P\beta}$ 22 Hz, $J_{P\beta,P\gamma}$ 22 Hz.

3'-N-Ethylamino-3'-deoxythymidine 5'-triphosphate (8) (ammonium salt). The synthesis was performed with the same molar quantities of **6** and other reagents and under the same conditions as in the case of 7 to give 8 (5 mg, 23%).

3'-N-Ethylamino-3'-deoxythymidine 5'-phosphite (9) (ammonium salt). Phosphorous acid (36 mg, 0.44 mmol) and N,N'-dicyclohexylcarbodiimide (93 mg, 0.45 mmol) was added to the solution of thoroughly dried **6** (60 mg, 0.22 mmol) in dry pyridine (20 ml) under stirring at room temperature. After 24 h the solution was treated with 100 ml of water, evaporated and reevaporated with toluene. The standard procedure of isolation using column (2 x 15 cm) with DEAE cellulose (HCO_3^-), eluent NH_4HCO_3 solution (0 → 0.1 M, total volume 1 l) allowed to get **9** (49 mg, 66%), R_f 0.2 (B). ^{31}P NMR (D_2O): δ 6.44d, $J_{P,H}$ 642 Hz. FAB mass spectrum: m/z 324 (MH^+).

Enzymology. We used [α-^{32}P]dATP, [α-^{32}P]dCTP, [γ-^{32}P]ATP with specific activities of 1000-3000 Ci/mmol (Radioisotope, Russia), dNTP (Sigma), BioGel A-1.5M (Bio-Rad Laboratories), T4 polynucleotide kinase, KFr from *E. coli* (Amersham), DNA polymerase β from rat liver [25], DNA polymerase α and ε from human placenta [3], reverse transcriptases from AMV (Omutninsk Chemical Plant, Russia) and HIV-1 [26], and a tetradecanucleotide as a primer. One unit of enzyme activity was defined as the amount catalyzing the incorporation of 1 nmol of four radioactive deoxynucleotides into DNA in 30 min.

Primer-template hybridization. The DNA of M13mp10 phage was isolated from the cultural fluid of the recipient K12XL1 *E. coli* strain. The primer (d(CCCAGTCACGACGT)) was labeled at the 5'-position by [γ-^{32}P]ATP using T4 polynucleotide kinase as described in [27]. Then M13mp10 DNA (+ chain) (0.5 μM) was annealed with 0.75 μM 5'-[^{32}P]-primer. The primer - template complex was purified by gel filtration on a BioGel A-1.5M microcolumn, prewashed by 10 mM Tris-HCl, pH 7.6 and 1 mM EDTA. The unlabeled primer-template complex for inhibition reactions was prepared as described above without gel filtration.

Template-dependent elongation of 5'-[^{32}P]- oligonucleotide catalyzed by DNA polymerases. Reaction mixtures (5 μl) contained 5'-[^{32}P]-labeled primer-template complex (0.05 pmole, 0.07 μCi), 60 μM each of dCTP, dGTP, and dATP, 6 μM dTTP, analogs **7** and **8** in various concentrations and (i) KFr (0.5 activity unit), 10 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 1 mM dithiotreitol, (ii) AMV RT or HIV RT (1 activity unit), 10 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 1 mM dithiotreitol, 40 mM KCl (for HIV RT glycerol (to 2%), and 0.05 mM Triton X-100 with various pHs were added), (iii) DNA polymerases α, ε or β (1 activity unit), 10 mM Tris-HCl, pH 7.4, 6 mM $MgCl_2$, and 0.4 mM dithiotreitol. The assay mixtures were incubated for 10 min at 37°C and

terminated by the addition of formamide (5 μl) containing dyes and 20 mM EDTA [27]. The reaction products were separated by electrophoresis in 12% denaturing polyacrylamide gel.

Some samples were additionally chased with a mixture of 400 μM dNTPs for 15 min at 37°C.

Inhibition of DNA synthesis catalyzed by DNA polymerases with compounds 7 and 8. Reaction mixtures contained the same components, unlabeled tetradecanucleotide at the concentrations as above, and 6 μM [α-^{32}P]dATP with a specific activity of 300 Ci/mmol instead of 60 μM dATP. The assay mixtures were incubated for 15 min at 37°C and terminated by the adding 1 ml of 0.5 M EDTA. The aliquots were placed on 0.5 x 1 cm strips of Whatman DE-81 paper, the strips were washed with 5% Na_2HPO_4, fixed by ethanol and assayed for radioactivity by liquid scintillation counting.

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Note in proof. A paper [28] concerning the synthesis of 3'-N-substituted 3'-amino-3'-deoxythymidine derivatives was published soon after we finished this study.

References

1. Horwitz, J.P.; Chua, J.; Noel, M.J. *J. Org. Chem.*, 1964, **29**, 2076-2078.
2. Chidgeavadze, Z.G.; Beabealashvilli, R.Sh.; Atrazhev, A.M.; Kukhanova, M.K.; Azhayev, A.V.; Krayevsky, A.A. *Nucl. Acids Res.*, 1984, **12**, 1671-1686.
3. Mozzherin, D.Yu.; Atrazhev, A.M.; Kukhanova, M.K. *Molec. Biology (Russian)*, 1992, **26**, 999-1010.
4. Lin, T.-S.; Chew, M.S.; McLaren C.; Gao, Y.-S.; Ghazzouli, I.; Prusoff, W.H. *J. Med. Chem.*, 1987, **30**, 440-444.
5. Fisher, P.H.; Lin, T.-S.; Prusoff, W.H. *Biochem. Pharm.*, 1982, **31**, 125-128.
6. Chen, M.S.; Woods, K.L.; Prusoff, W.H. *Molecular. Pharm.*, 1984, **25**, 441-445.

7. Krayevsky, A.A.; Kukhanova, M.K.; Atrazhev, A.M.; Chidgeavadze, Z.G.; Beabealashvilli, R.Sh. *Molec. Biol. (Russian)*, 1987, **21**, 33-38.
8. Krayevsky, A.A.; Kukhanova, M.K.; Atrazhev, A.M.; Dyatkina, N.B.; Papchikhin, A.V.; Chidgeavadze, Z.G.; Beabealashvilli, R.Sh. *Nucleosides & Nucleotides*, 1988, 7, 613-617.
9. Wigerinck, P.; Van Aerschot, A.; Janssen, G.; Claes, P.; Balzarini, J.; De Clerck, E.; Herdewijn, P. *J. Med. Chem.*, 1990, 33, 868-873.
10. Maillard, M.; Farai, A.; Frappier, F.; Florent, J.-L; Grierson, D.S.; Monnerret, C.; *Tetrahedron Lett.* 1989, 30, 1955-1958.
11. De Clercq, E.; Van Aerschot, A.; Herdewijn, P.; Baba., M.; Pauwels, R.; Balzarini, J. *Nucleosides & Nucleotides*, 1989, **8**, 659-671.
12. Jasko, M.V.; Atrazhev, A.M.; Mozzherin, D.Yu.; Novicov, N.A.; Fedorov, I.I.; Krayevsky, A.A. *Bioorg. Chem. (Russian)*, 1992, **18**, 299-301.
13. Vial, J-M.; Johansson, N.G.; Chattopadhyaya, J. Thesises of 9th International Round Table Nucleosides, Nucleotides & their Biological Applications, July 30-August 3, 1990, Uppsala, Sweden, p.174.
14. Kazmina, E.M.; Fedorov, I.I.; Bezchinsky, Ya.E.; Kiseleva, N.V.; Novicov, N.A.; Galegov, G.A.; Arzamastsev, A.P. *Chem. Pharm. Journal (Russian)*, 1989, 23, 1217-1223.
15. Morr, M.; Ernst, L. *Chem. Ber.*, 1979, 112, 2815-2828.
16. Karamov, E.V.; Lukashov, V.V.; Tarussova, N.B.; Kornilayeva, G.V.; Rodova, M.A.; Kukhanova, M.K.; Krayevsky, A.A. *Molec. Biology (Russian)*, 1990, 24, 1695-1701.
17. Tarussova, N.B.; Khorlin, A.A.; Krayevsky, A.A.; Korneyeva, M.N.; Nossik, D.N.; Kruglov, I.V.; Galegov, G.A.; Beabealashvilli, R.Sh. *Molec. Biol. (Russian)*, 1989, 23, 1716-1723.
18. Ludwig, I. *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1981, **16**, 131-133.
19. Haasnoot, C.A.G; de Leeuw, F.A.A.; de Leeuw H.P.M.; Altona, C.J. *Org. Magn. Res.* 1981, 15, 43-52.
20. Rinkel, L.; Altona, C.J. *Biomol. Struct. Dynam.* 1987, **4**, 621-649.
21. Young, B.V.; Tollin, P.; Wilson, H.R. *Acta Cryst.*, 1969, B25, 1423-1432.
22. 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.
23. Gurskaya, G.V.; Tsapkina, E.N.; Lindeman, S.V.; Struchkov, Ju. T.; Krayevsky, A.A. *Dokl. Acad. Nauk SSSR*, 1988, **303**, N6, 1378-1382.

24. Harte, W.H.; Starret, J.E.; Martin, J.S.; Mansuri, M.M. *Biochem. Biophys. Res. Commun.*, 1991, **175**, 298-304.
25. Atrazhev, A.M.; Kukhanova, M.K. *Bioorg. Chem. (Russian)*, 1985, **11**, 1627-1635.
26. 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.
27. Maniatis, T.; Fritsch, E.E.; Sambrook, J. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press. NY. 1989.
28. Celewicz, L., Urjasz, W., Golankiewicz, K. *Nucleosides & Nucleotides*, 1993, **12**, 951-966.

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