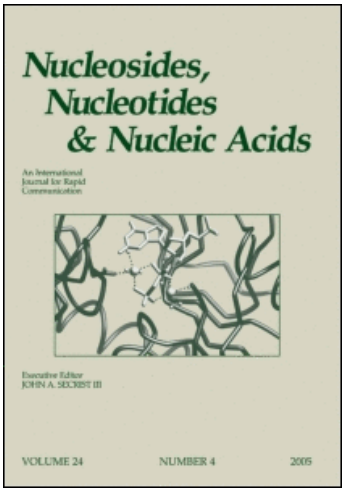


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Substrate Properties of C'-Methylnucleoside and C'-Methyl-2'-deoxynucleoside 5'-Triphosphates in RNA and DNA Synthesis Reactions Catalysed by RNA and DNA Polymerases

S. N. Mikhailov[a]; N. Sh. Padyukova[a]; Yu P. Lysov[a]; L. P. Savochkina[b]; Z. G. Chidgeavadze[b]; R. Sh. Beabealashvilli[b]
[a] Engelhardt Institute of Molecular Biology, Academy of, Sciences of the USSR, Moscow [b] All-Union Cardiology Research Center, Academy of Medical Sciences of the USSR, Moscow

To cite this Article Mikhailov, S. N. , Padyukova, N. Sh. , Lysov, Yu P. , Savochkina, L. P. , Chidgeavadze, Z. G. and Beabealashvilli, R. Sh.(1991) 'Substrate Properties of C'-Methylnucleoside and C'-Methyl-2'-deoxynucleoside 5'-Triphosphates in RNA and DNA Synthesis Reactions Catalysed by RNA and DNA Polymerases', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 339 — 343
To link to this Article: DOI: 10.1080/07328319108046474
URL: http://dx.doi.org/10.1080/07328319108046474

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 339-343 (1991)

SUBSTRATE PROPERTIES OF C'-METHYLNUCLEOSIDE AND C'-METHYL-2'-DEOXYNUCLEOSIDE 5'-TRIPHOSPHATES IN RNA AND DNA SYNTHESIS REACTIONS CATALYSED BY RNA AND DNA POLYMERASES

S.N.Mikhailov, N.Sh.Padyukova, Yu.P.Lysov, L.P.Savochkina*, Z.G.Chidgeavadze*, and R.Sh.Beabealashvilli*

Engelhardt Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, 117984
*All-Union Cardiology Research Center, Academy of Medical Sciences of the USSR, Moscow, 121552

ABSTRACT. C'-Methyl derivatives of NTP and dNTP were prepared and their substrate properties were investigated in RNA and DNA synthesis reactions.

The present work is a sequel to earlier experiments in which we synthesized functionally competent analogs of nucleosides and their phosphoric esters as well as studied their physico-chemical and biological properties[1]. These analogs contain all the functionalities of natural compounds, i.e. all the possible binding sites for the enzymes. Here we sum up the data on the substrate properties of C'-methylribo (and 2'-deoxyribo)nucleoside 5'-triphosphates (**1a-4a** and **1b-3b**) in the reactions of RNA and DNA synthesis catalysed by different RNA and DNA polymerases.

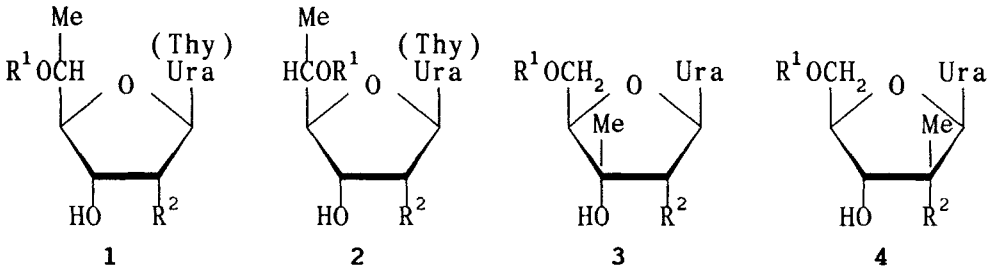
The C'-methylribonucleosides synthesized earlier (**1c-4c**)[1] were converted into the corresponding 5'-triphosphates (**1a-4a**) and 2'-deoxyderivatives (**1d-3d**)[2,3] and then into 5'-triphosphates (**1b-3b**). Their substrate properties were studied in the reactions of RNA and DNA synthesis catalysed by *E.coli* Klenows fragment DNA polymerase I and RNA polymerase, terminal deoxynucleotidyl transferase (TDT) and DNA polymerase α from calf thymus, and avian myeloblastosis virus reverse transcriptase (RT). RNA was synthesized on T7 ΔDIII

TABLE 1. Substrate properties of C′-methylnucleoside 5′-triphosphates in RNA and DNA synthesis reactions.

Enzymes	C′-methylnucleoside 5′-triphosphates			
	1a	**2a**	**3a**	**4a**
RNA-polymerase	substrate	substrate	terminator	substrate
TDT	*	*	*	*
	C′-methyl-2′-deoxynucleoside 5′-triphosphates			
	1b	**2b**	**3b**	
DNA-polymerase I	substrate	substrate	inhibitor	
DNA-polymerase α	substrate	substrate	inhibitor	
RT	substrate	substrate	inhibitor	
TDT	*	substrate	*	

*NTP analogs were not transformed

phage DNA in the presence of CpA which allows one to initiate RNA synthesis from an A1 promoter (the experiment is described in detail elsewhere[4,5]). Reactions catalysed by DNA polymerases were carried out with an equimolar complex of M13 mp10 phage DNA and with a $[p^{32}]$dCCCAGTCACGACGT labeled primer. The same primer was used in the synthesis catalysed with TDT without a template. The results are listed in Table 1.



a: $R^1 = P_3O_9H_4$, $R^2 = OH$ **b**: $R^1 = P_3O_9H_4$, $R^2 = H$

c: $R^1 = H$, $R^2 = OH$ **d**: $R^1 = R^2 = H$

It would be relevant to note that 5'-triphosphates of 5'-C-methylnucleosides belonging to the D-allo and L-talo series (**1a** and **2a**) are integrated into a growing RNA chain by RNA polymerase with a different effectiveness[4] whereas 5'-triphosphates of 3'-C-methylnucleosides are terminating substrates for this enzyme and can be used to sequence nucleic acids[6]. **1b** and **2b** are substrates for all the studied DNA polymerases and the corresponding modified nucleoside residues can be integrated into the middle of a DNA chain. On the contrary, **3b** cannot be incorporated into a growing DNA chain and is an inhibitor of this reaction. In the reaction catalysed by TDT, **1b** is not a substrate while **2b** is built into the 3'-end of the oligonucleotide, and the formed dpCCCAGTCACGACGT5'MeT cannot be extended readily when dTTP is added. It should be noted that both ribo-UTP derivatives **1a** and **2a** are not substrates for this enzyme.

The reactivity of hydroxyls and conformational properties are modified when a methyl group is incorporated into a nucleoside molecule. The presence of bulky methyl groups may induce steric hindrances due to intermolecular and intramolecular collisions when the substrate is fixed in a certain conformation in the enzyme-substrate complex.

In order to detect intramolecular contacts between a methyl group and a heterocyclic base conformational analysis of nucleosides and their analogs using force-field method in the approximation of "rigid" bond lengths was carried out. The conformational energy maps (the χ- P dependence versus the potential energy E) are nearly identical for 5'-C-methylnucleosides and natural nucleosides when the exocyclic $HOCH_2$ group has a fixed gauche-gauche orientation ($\gamma=60^{o}$). Differences appear when the methyl group of an analog is arranged above the furanose cycle[7]. Then the heterocyclic base will collide with the methyl group both in *syn* and *anti* regions. The energetically forbidden conformation will be defined as a conformation when the following condition is met: $E_{analog} - E_{natural\ nucleoside} > 10$ kcal/mol. Table 2

Table 2. Energetically forbidden conformations of 2′-C- and 3′-C-methyluridine (E_{analog}-$E_{natural\ nucleoside}$>10 kcal/mol)

Conformation *	Energetically forbidden regions** of glycoside angle χ values	
	2′-C-methyluridine	**3′-C-methyluridine**
N-region		
P=0° $^{3}_{2}T$	80–185°, 265–360°	10–125°, 195–295°
P=18° ^{3}E	65–190°, 260–360°	10–115°, 195–290°
P=36° $^{3}_{4}T$	55–190°, 250–360°	20–100°, 210–270°
P=54° $_{4}E$	55–185°, 240–360°	35–85°, 230–245°
S-region		
P=126° $_{1}E$	45–145°, 235–325°	–
P=144° $^{2}_{1}T$	35–145°, 235–320°	–
P=162° ^{2}E	35–145°, 215–315°	–

*Calculated at fixed values of the angle γ=60°. The methyl group collides within the entire range of glycoside angles χ in the $^{2}_{3}T$ conformer (P=180°) for 2′-C-methyluridine.

**For the remaining regions $E_{analog} \simeq E_{natural\ nucleoside}$.

presents these regions of χ angles for the main conformers of 2′-C- and 3′-C-methyluridine[5]. Only N-conformers of 3′-methylnucleosides have energetically forbidden conformations whereas no intramolecular collisions occur in S-conformers. The energy barrier of a *syn-anti* conversion rises noticeably when a methyl group is introduced in the 2′-position.

When RNA polymerase is used, **3a** acts as a terminating substrate and **4a** can substitute for UTP in the reactions of RNA synthesis. Therefore, its conformations in enzyme-substrate complexes do not acquire forbidden states. Hence, possible χ values for the substrate are in the *anti* region (150–240°), which is consistent with the conformations of RNA double helices in the A form[8].

The conformation of a carbohydrate residue ought to be known in order to specify the substrate conformation. For the N-family typical of RNA double helices[8], an allowed substrate conformation in the enzyme-substrate complex is confined to the χ angle range of 185 to 195° according to the calculated data for 2'-C- and 3'-C-methyluridines.

The finding that **3b** cannot act as a substrate in the reactions of DNA synthesis may be associated with steric hindrances within the χ angle range of 210 to 270° in the N-family of conformers.

The application of this approach to other enzymes of nucleic acids biosynthesis has made it possible to specify the conformation of a substrate in the course of its enzyme-catalysed transformation.

REFERENCES

1. S.N.Mikhailov, *Nucleosides and Nucleotides*, 1988, 7, 679.
2. N.Sh.Padyukova, M.V.Fomitcheva, S.N.Mikhailov, M.Janta-Lipinsky, *Bioorgan.Khim.(USSR)*, 1990, 16, 675.
3. S.N.Mikhailov and M.V.Fomitcheva, *Bioorgan.Khim. (USSR)*, 1990, 16, 699.
4. V.A.Aivazashvilli, S.N.Mikhailov, N.Sh.Padyukova, R.Sh. Beabealashvilli, M.Ya.Karpeisky, *Mol.Biol. (USSR)*, 1987, 21, 1080.
5. L.P. Savochkina, T.V. Sviriyeva, L.N. Beigelman, N.Sh. Padyukova, D.A.Kuznetsov, Yu.P. Lysov, S.N. Mikhailov, R.Sh.Beabealashvilli, *Mol.Biol. (USSR)*, 1989, 23, 1700.
6. V.A.Aivazashvilli, S.N.Mikhailov, N.Sh.Padyukova, M.Ya. Karpeisky, R.Sh.Beabealashvilli, *Bioorgan.Khim. (USSR)*, 1986, 12, 708.
7. S.N.Mikhailov, S.V.Meshkov, D.A.Kuznetsov, Yu.P.Lysov, E.Sh.-B. Gorelik, M.V.Fomitcheva, L.N.Beigelman, N.Sh. Padyukova, *Bioorgan.Khim.(USSR)*, 1989, 15, 969.
8. W.Saenger, "*Principles of Nucleic Acid Structure*", Springer Verlag, 1984, Berlin.