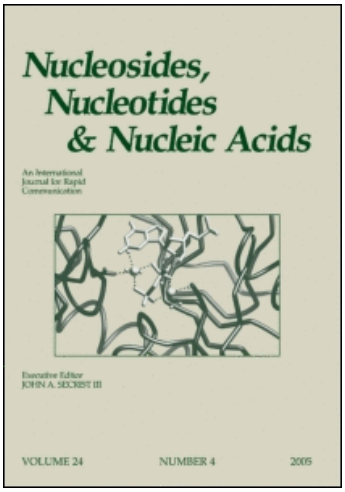

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Possibility for the Existence of a General Conformational Motif in the Active Sites of Enzymes Which are Involved in Nucleic Acids Metabolism

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OPINION

POSSIBILITY FOR THE EXISTENCE OF A GENERAL CONFORMATIONAL MOTIF IN THE ACTIVE SITES OF ENZYMES WHICH ARE INVOLVED IN NUCLEIC ACIDS METABOLISM.

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ABSTRACT: Many different modified nucleosides and nucleotides with conformationally restricted partly flattened sugar residues are analyzed as substrates or inhibitors of several groups of enzymes of nucleic acid metabolism. A detailed examination of the sugar moiety of large group of modified nucleosides showed that there is a striking conformational similarity, *i.e.*, they are flattened. We propose herein a hypothesis which can represent a general conformational elements in the structure of the active sites of several different groups of enzymes. This proposal envisions that during the enzymatic process natural substrates should reflect these flattened conformations. This hypothesis allows computation of conformational analyses of the enzyme actives centers as well as the design of new actively metabolized modified nucleosides.

INTRODUCTION

After a detailed examination of the available information on a large group of nucleosides and nucleotides which act as substrates or inhibitors of many different types of enzymatic reactions, we found that striking similarity exists among the nucleosides in terms of the sugar conformation. We found that, viewed from such similarity, many of the seemingly complicated and unrelated enzyme reactions can now be readily accounted for and, indeed, such an "overview" may help medicinal

chemists in the design, synthesis and prediction of biologically active nucleosides and nucleotides.

The carbohydrate moiety of nucleic acids components has a strong influence on the conformational state for several reasons. The sugar residue is the most conformationally flexible part of the nucleosides and nucleotides while the nucleobases are conformationally rigid. They can only rotate about the glycosyl bond. The sugar conformation, therefore, defines the general conformational state of nucleoside and nucleotide. This role of the sugar residue is realized both in monomeric and in polymeric molecules. In the latter case, the parameters for DNA and RNA duplexes are mainly defined by the conformation of the sugar residues in their chains.[1] For interaction with many different enzymes, the nucleic acid should have the possibility of binding to the recognition areas of the enzymes, and thus they would have a chance to change their conformation if it is required for interaction.

There are two viewpoints that may be suggested for the structure of the active sites recognition area of enzymes which catalyze the chemical transformations of the nucleic acids. One viewpoint is that each group of enzymes has its own conformational characteristics for its recognition site, and it differs from the corresponding characteristics of other groups of enzymes. The second viewpoint is based on the postulate that many groups of enzymes that catalyze the transformations of nucleic acids have one general conformational motif for their recognition site for the sugar residues of the substrates, because these enzymes are derived from one original enzyme. During the functional divergence, these enzymes have kept the general conformational element of the recognition site for the ribosyl residue.

It is well known that the ribo- or 2'-deoxyribo-furanose residue can possess two extreme twist conformations, north (N) and south (S), and several sets of intermediate conformations between these two. One of the problems with natural nucleic acids is that interconversion among conformers require low energy (<1 kcal/mol)[2] so that it is impossible to determine with which particular conformation the nucleoside or nucleotide is bound to the active sites of the enzymes. The other problem is the way to present the conformational change in these compounds *during the catalytic processes*, since there are no data available about the degree of

conformational changes in the dynamic state. The solution of all these problems requires several different methods of studies, for example, X-ray analysis, NMR spectroscopic study, *etc.*

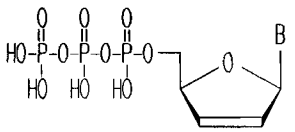
One of the most promising methods to our knowledge would be to synthesize nucleic acids and their analogues with decreasing conformational flexibility in the sugar residue and study their biological and physicochemical properties.

Many X-ray data on crystalline structures of natural and modified nucleosides are now available. For example, thymidine,[3] 3'-azido-3'-deoxythymidine,[4] 3'-amino-3'-deoxy-thymidine,[5] 3'-deoxythymidine,[6] 3'-fluoro-3'-deoxythymidine,[7] *etc.* There are no strong conformational similarities in crystalline structures among these nucleosides. Moreover, some of them are crystallized in several forms. Therefore, it is extremely difficult, if not impossible, to predict the conformational state of these nucleosides which are bound to the active centers of the enzymes. A speculation was published that the activity of several modified nucleosides in a total enzymatic cascade: "nucleoside -> nucleoside 5'-monophosphate -> nucleoside 5'-diphosphate -> nucleoside 5'-triphosphate -> DNA chain" involves the S- (not N-) conformation and *gauche* (diaxial) orientation of the substituents around the C5'-O5'-bond.[8,9] This speculation was based on the observation that all the active nucleosides possessed the S-conformation in the crystalline state. The N-conformation was found in a crystalline form of 3'-amino-3'-deoxythymidine which is inactive against HIV-1.[10] This discovery is in contradiction to this hypothesis, because this nucleoside can be phosphorylated and incorporated into DNA chains in many cell types[11,12] as well as in cell free systems by different DNA polymerases.[13,14] Herdewijn *et al.*[15] concluded recently that there is little direct correlation between conformation in crystalline structure and activity.

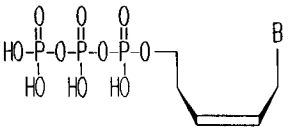
1. *Modified nucleoside triphosphates with conformationally restricted sugar moiety as chain terminators of DNA polymerases.*

During 1985-1986, using an NMR technique, it was found that in complexes with DNA polymerase I from *Escherichia coli*, the natural 2'-deoxynucleoside 5'-triphosphates (dNTP) are in a flattened sugar conformation.[16,17] One year later, 3'-

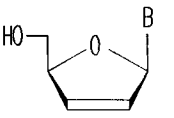
deoxy-2',3'-didehydrothymidine 5'-triphosphate (**Ia**, d_4TTP) was shown to be a very strong chain terminator for several DNA polymerases.[18,19] The mechanism of action of **Ia** is to interact with DNA polymerases such as DNA dependent DNA polymerases, RNA dependent DNA polymerases (reverse transcriptases) or template independent DNA polymerase (terminal deoxynucleotidyl transferase), and to be incorporated into the 3' end of DNA chains and terminate the subsequent elongation. Affinity of the d_4NTP (**I**) to DNA polymerases was investigated in detail for d_4TTP (**Ia**). It was found that **Ia** has high affinity to HIV reverse transcriptase (K_i=O.008-0.032 μM in different testing systems; for dTTP K_m=5-6 μM),[20-23] to human DNA polymerase γ (K_i=0.0035 μM, for dTTP K_m=0.63)[24], to human and mammalian DNA polymerases ß-type,[18,19,23] reverse transcriptases of avian myeloblastosis virus,[18,19] Rous sarcoma virus,[18,19] murine leukemia virus[24] and mammalian terminal deoxynucleotidyl transferase.[18,19] The K_i values measured in polymerization reactions for modified nucleoside 5'-triphosphates were compared with the Michaelis constant (K_m) for natural substrate. The affinity of d_4TTP was very low only to human and mammalian DNA polymerase α.[18,19,23,24] The same and even higher affinity are shown by **IIa-c**, especially to DNA polymerase α.[25] The termination property of d_4NTP for some DNA polymerases have been demonstrated by St. Clair *et al.*[26] and Elwell *et al.*[27] The double bond in **I** makes the sugar residue more flattened and, con-sequently, it was proposed that **I** reflected the natural substrate dNTP conformation in the transition state during the reaction catalyzed by DNA polymerases. The X-Ray analyses of the corresponding 2',3'-dideoxy-2',3'-didehydronucleosides **IIIa**,[28,29] **IIIb**,[30] **IIIc**[31] and **IIId**[32] showed that C1', C2', C3' and C4' atoms in the ring are almost, but not absolutely, coplanar, and the O4' atom is slightly out of plane (*endo*-configuration).



Ia B = Thy
Ib B = Cyt



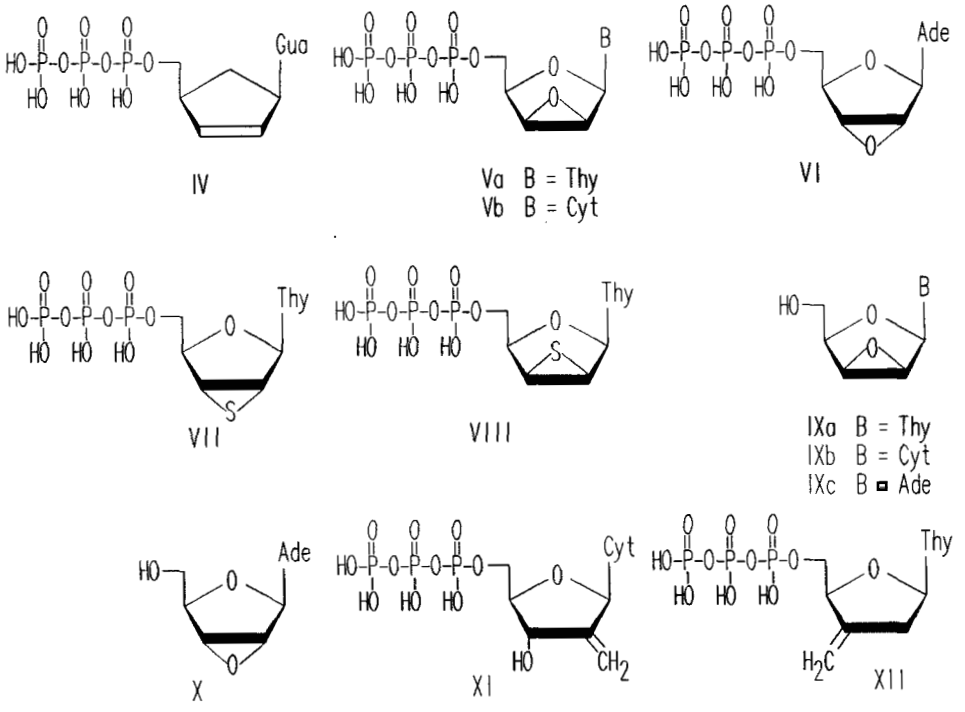
IIa B = Thy
IIb B = Cyt
IIc B = Ade



IIIa B = Thy
IIIb B = Cyt
IIIc B = Ade
IIId B = Gua

Later, the triphosphates **Ib**[24] and the carbocyclic analogue of 2',3'-dideoxy-2',3'-didehydroguanosine (**IV**, carbovir)[33] were found to have chain termination properties.

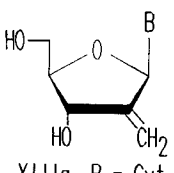
Additional compounds were studied as conformationally restricted substrates for DNA polymerases. One set of compounds includes 2',3'-lyxoanhydronucleoside 5'-triphosphates (Va,b),[34,35] 2',3'-riboanhydroadenosine 5'-triphosphate (VI),[34,35] 2',3'-epithioribo-2',3'-dideoxythymidine (VII) and 2',3'-epithio-β-D-lyxofuranosylthymine (VIII)[36] which were shown to be chain terminators for DNA polymerases. The X-ray analyses of 2',3'-lyxoanhydrothymidine (IXa)[37,38] and 2',3'-riboanhydroadenosine (X),[39] the nucleoside portion of Va and VI, respectively, revealed that C1', C2', C3' and C4' are nearly coplanar. Another set of compounds that bear the chain terminating property for DNA polymerases are 2'-methylidene-2'-deoxycytidine 5'-triphosphate (XI)[40] and 3'-methylidene-2',3'-dideoxythymidine 5'-triphosphate (XII)[41]



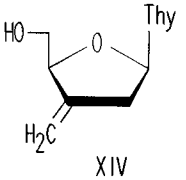
which also contain a partly planar sugar residue. Compound XI is known to act as a chain terminator for human DNA polymerases,[40] although the molecular mechanism of its action has not been published yet. In the sugar residue of the corresponding nucleosides XIII and XIV, C1', C2' and C3' in the former[40] and C2',C3' and C4' in the latter[41] are, again, nearly coplanar as determined by X-ray analyses. The triphosphate XII has been shown to act as a chain terminator for several DNA polymerases.[41]

More recently, O[4']-nor-2',3'-dideoxy-2',3'-didehydronucleoside 5'-triphosphates (II) are found to have potent chain terminating property for a variety of DNA polymerases including DNA dependent DNA polymerases of human, mammalian and viral origin, viral reverse transcriptases and calf thymus terminal deoxynucleotidyl transferase.[25] Concentrations of II required for 50% inhibition (IC_{50}) of DNA synthesis in some cases were even less than the concentrations of natural substrates. This means that their affinity to the active site of DNA polymerases is very high.

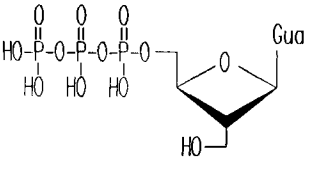
In 1991, the effects of oxetanocin-G 5'-triphosphate (XV) on DNA synthesis were reported.[41] Compound XV was found to inhibit DNA synthesis catalyzed by DNA poly-merases of cytomegalovirus and herpes simplex type 2 virus. It also inhibits DNA synthesis catalyzed by human DNA polymerases, albeit to a lesser degree. The tetrose conformation in XV is slightly deviated from planarity. The conformational flexibility of this glycone is limited due to its chemical nature. Although XV is shown to compete with dGTP in the synthesis catalyzed by the above DNA polymerases, the



XIIIa B = Cyt
XIIIb B = Ura



XIV



XV

exact molecular mechanism of action is not completely elucidated. Table 1 lists the DNA polymerases which accept modified nucleosides with conformationally restricted sugar residue as chain terminators.

Table 1. DNA polymerases and their chain terminators

Enzyme		Substance
Human and mammalian:		
DNA polymerase	α	XI[38*], II[25], XV[25*],
	ß	I[19], II[25], VI[34], VII[35]
	γ	I[24]
	ϵ	II[25]
Terminal deoxynucleotidyl transferase		I[19], II[25], V[34], VI[34]
Viral reverse transcriptases		
HIV		I[26,27], II[25], IV[33], VII[36], XII[41]
Avian myeloblastosis virus		I[19], II[25], IV[33], V[34], VI[34], VII[36], VIII,[36] XII[41]
Rausher moloney leukemia virus		I[25]
Rous sarcoma virus		I[19]
Human hepatitis B virus		I[43]
DNA polymerases of viruses:		
Herpes simplex 1 virus		II[44]
Cytomegalovirus		II[44], XV[42]
Bacterial DNA polymerases:		
I from *E.coli*		I[19], VI[34]
Thermus aquaticus		I[45]
Archebacterium		
Sulpholobus acidocardarius		VI[46]

* Total preparation of human DNA polymerases.

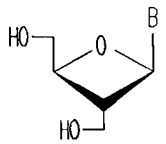
Unfortunately, many investigations with DNA polymerases are made on qualitative or semiquantitative level and we are not able to compare K_m or V_{max} for the listed compounds. There are only few exceptions. So K_i/K_m ratio for XV and for several viral and human DNA polymerases is between 0.6-3.9,[36] where the K_m is the Michaelis constant for dGTP with which XV competes in these reactions. It means that this modified nucleoside 5'-triphosphate has affinity nearly the same as dGTP to the DNA polymerases.

2. *Modified nucleosides with conformationally restricted glycones with antiviral and anticancer properties.*

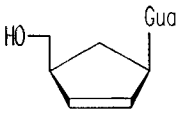
There are many other conformationally restricted nucleosides which have been found to inhibit the overall process of DNA biosynthesis in cells. The molecular

mechanisms of inhibition of enzymes are yet to be determined. Nevertheless, the properties of conformationally restricted nucleosides summarized as above for DNA polymerases may illustrate the molecular targets of their actions. First of all, it can be pointed out that among these compounds, **IIIa**,[47] **IIIb**,[47] **IXb**[48] and carbovir **XVII**[49] are potent inhibitors of HIV reproduction. All these compounds can be phosphorylated to their 5'-triphosphates (**Ia**, **Ib**, **IV** and **Vb**, respectively), which terminate the DNA chain elongation. In the process of phosphorylation they have to be the substrates for the corresponding phosphorylating enzymes. Similar properties are known for **XVI**[42] with exception that these two compounds are better substrates for the herpes encoded thymidine kinase than for cellular kinases.

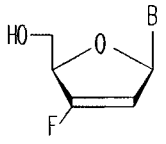
There are many modified nucleosides that block the viral replication (first of all - HIV reproduction, for which studies of such compounds are more advanced) by a mechanism apparently similar to the one described above. This group of nucleosides includes 3'-fluoro-2',3'-dideoxy-2',3'-didehydro-nucleosides (**XVIII**)[51] with adenine, cytosine and thymine bases, 2'-fluoro-2',3'-dideoxy-2',3'-didehydrocytidine (**XIX**)[51], 2',3'-α-methylene-2',3'-dideoxycytidine (**XX**)[52], oxetane derivative of 4'-hydroxymethylthymidine (**XXI**)[53], 9-(2-azido-2-deoxy-ß-D-*erythro*-oxetanosyl)adenine (**XXIIa**)[54], 9-(ß-D-*erythro*oxetanosyl)adenine (**XXIIb**)[55] and 9-(2-deoxy-ß-D-*erythro*-oxetanosyl)adenine (**XXIIc**)[55], 9-(ß-D-*threo*-oxetanosyl)adenine (**XXIII**)[55], 9-(t-2,c-3-dihydroxymethyl-r-1-cyclopropyl)-9H-adenine (**XXIV**)[56], cytallene and adenallene (**XXV**)[57]. Compounds **III**, **IX-X**, **XIV** and **XVII-XXI** bear a five-membered sugar residue, and compounds **XVI** and **XXII-XXIII** a four-membered sugar. It should be noted that the sugar conformation of all these nucleosides are rather rigid. Some compounds do not contain any functional group on the carbo-hydrate moiety other than the heterocyclic base and hydroxyl group. The latter takes part in phosphorylation by the enzyme. Thus, it appears that the most critical element in the molecule for binding at the active sites of the corresponding



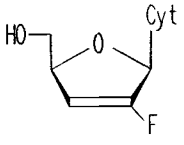
XVIa B = Gua
XVIb B = Ade



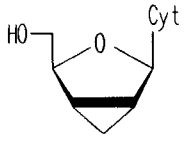
XVII



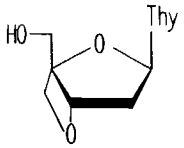
XVIIIa B = Cyt
XVIIIb B = Ade
XVIIIc B = Thy



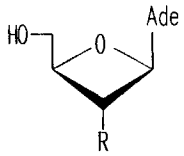
XIX



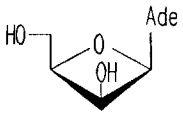
XX



XXI

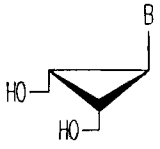


XXIIa R = N_3
XXIIb R = OH
XXIIc R = H

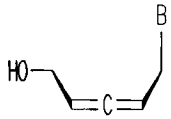


XXIII

enzymes is the conformationally restricted sugar more to that could properly place the base and the hydroxymethyl group to be phosphorylated onto the conformationally controlled positions in the active site.



XXIV



XXV B = Ade, Cyt

Table 2 shows some data on the substrate properties of certain modified nucleosides with respect to different enzymes. As in the case of the data on DNA polymerases there are little quantitative analysis data available at present.

3. *Modified ribonucleosides with conformationally restricted glycone.*

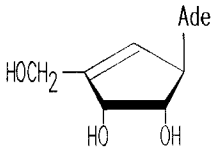
There is another large group of substrate analogues and inhibitors of enzyme-catalyzed transformations of ribonucleosides and ribonucleotides, which includes many ribonucleoside derivatives with limited conformational flexibility in the sugar moiety. The most studied among them are substrate analogues and inhibitors of the two closely related enzymes, S-adenosyl-methionine synthetase (Ado-Met synthetase) and S-adenosyl-L-homo-cysteine hydrolase (Ado-Hcy hydrolase). These two enzymes are responsible for the level of S-adenosyl-methionine (Ado-Met) in the cells and for a large set of Ado-Met dependent reactions. Therefore, substrate properties of some adenosine analogues open a large set of enzymatic reactions, in which these compounds can take part.

The antibiotic, neplanocin A (NpcA, XXVI), isolated from the culture filtrate of *Ampullariella regularis,* is the most investigated among the compounds of this

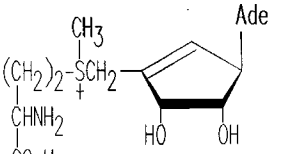
Table 2. Modified nucleosides as substrates for different enzymes

Compound	Enzyme
IIIa	Human Trd-kinase[58]; human and *E.coli* dTrd phosphorylase[59]
Vb	human dCyd-kinase[60]
IXc	calf thymus Ado-deaminase[61]
XIIIa	*E.coli* ribonucleoside diphosphate reductase[62] bovine liver Ado-Hcy hydrolase[62]
XIIIb	*E.coli* ribonucleoside diphosphate reductase[62]
XIIIc	human dCyd kinase[63], human Ado-deaminase[63] human Ado-kinase[63]
XXV	human dCyd-kinase[62]

type.[64] This antibiotic **XXVI** is shown to be a substrate analogue in many reactions of phosphorylation which convert NpcA to its 5'-mono-, 5'-di- and 5'- triphosphates.

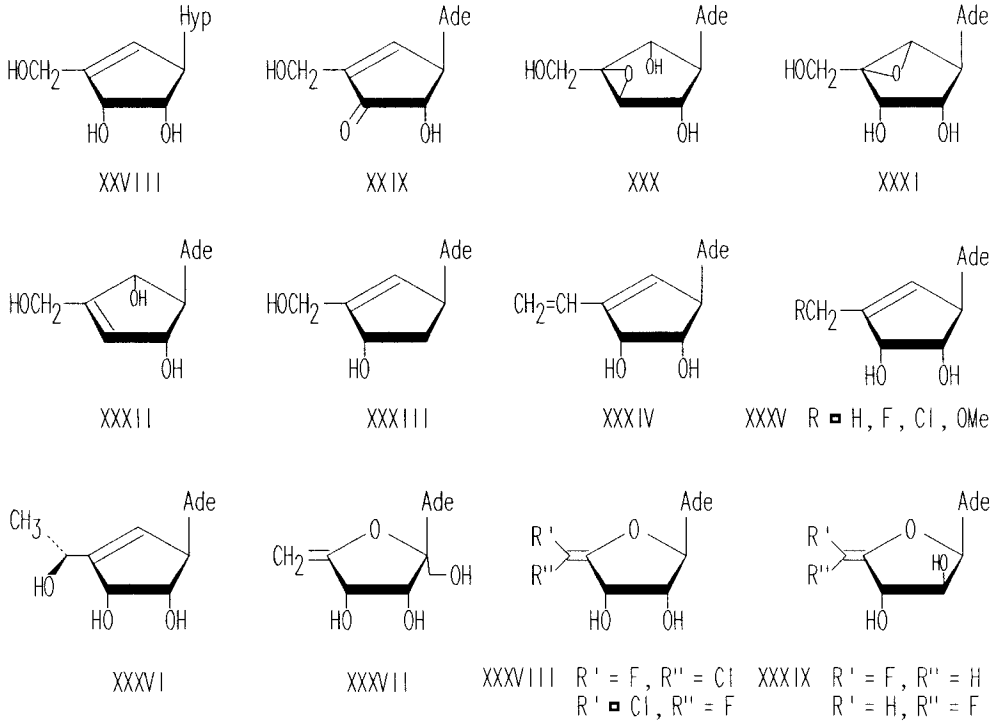


XXVI NpcA



XXVII

The monophosphate portion of the last compound can then be incorporated into RNA chain by the catalysis of human (and mammalian) RNA polymerases.[65] NpcA 5'-triphosphate can undergo AdoMet synthetase-catalyzed transformation reaction to give NpcA-methionine (XXVII). This product XXVII is a potent inhibitor of a wide spectrum of AdoMet dependent methyltransferases which catalyze methylation of many biomolecules including both low molecular compounds (*e.g.*, neurotransmitters, histamine, norepinephrine, *etc.*) and macromolecules (proteins, lipoproteins, nucleic acids).[66] In addition, NpcA-methionine (**XXVII**) is a substrate for catechol-O-methyltransferase.[67] **Neplanocin A also exhibits a strong inhibitory activity against AdoHcy hydrolase (Ki = 8.4 nM)[68,69] which catalyzes hydrolysis of AdoHcy into adenosine and homocysteine. Neplanocin A (XXVI) can be deaminated to**

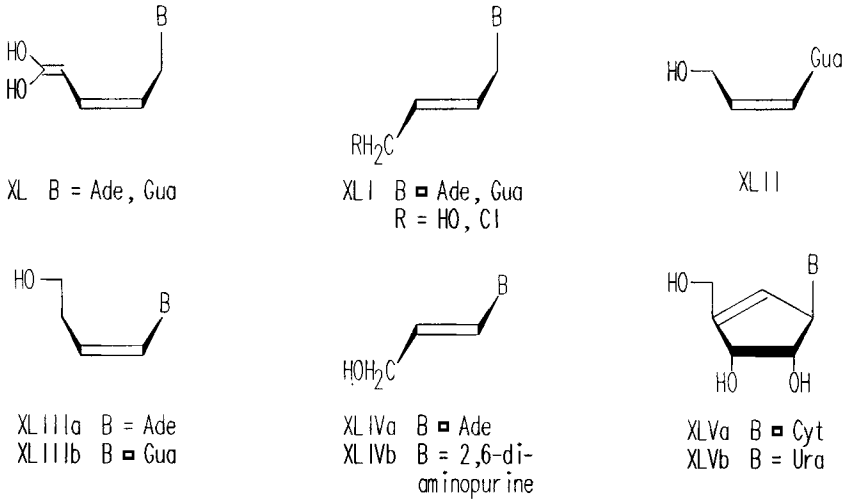


neplanocin D (**XXVIII**) by adenosine deaminase.[65] It can also be oxidized by AdoHcy *hydrolase* to give **XXIX**.[70,71] It should be noted that the cyclopentenyl ring in **XXVI** in crystalline structure deviates only slightly from planarity.[64]

It is apparent that all the reactions discussed above can be catalyzed by the same enzymes in the reverse directions, and it seems that all structures **XXVI-XXVIII** can be both products and substrates of the corresponding enzymatic reactions. Enzyme inhibitor activity is also observed for neplanocins B (**XXX**), C (**XXXI**), F (**XXXII**) and 2'-deoxyneplanocin A (**XXXIII**).[72,73]

Compounds **XXXIV-XXXVI** belong to a group of inhibitors of DNA and RNA viral replications.[74] In spite of the absence of hydroxyl group in the 5'-position, these molecules are capable of binding to the active sites of enzymes and block virus replic-ation. Angustomycin (**XXXVII**) and related adenine nucleosides **XXXVIII-XXXIX**[75] constitute additional examples of 5'-substituted nucleosides with limited conformational flexibility in the carbohydrate moiety. All these nucleosides inhibit mammalian Ado-Hcy hydrolase competitively with respect to the natural substrate.

Also, several acyclic unsaturated nucleoside analogues, such as **XL-XLIV**, have been shown to possess anticancer, antiviral, and sometimes antibacterial activities.[76] Nucleosides **XLI, XLIIIa** and **XLIVa** are competitive inhibitors of human adenosine



these compounds contain a double bond in the carbohydrate moiety in the 2',3'- or deaminase.[77] All 1',2'-position.

The cytosine analogue of neplanocin A, **XLVa** has a wide spectrum of antiviral activity.[78,79] The analogue **XLVa** is converted into its 5'-triphosphate in the cell, and then incorporated into the RNA chains.[79,80] The major mode of biological activity of **XLVa**-TP at submicromolar concentrations is inhibition of UTP aminotransferase which catalyzes the conversion of UTP to CTP reaction in the *de novo* pathway in mammalian cells.[80] This analogue is also a weak substrate for cytidine deaminase.[80] The metabolites of **XLVa** are also found to inhibit ribonucleoside diphosphate reductase and DNA polymerases.[79,80] Thus, **XLVa** as well as its deaminated metabolite, cyclopentenyluracil (**XLVb**) are inhibitors of mammalian uridine kinase.[79]

Table 3 summarizes enzymes that interfere with neplanocin A and analogues.

In most of these nucleosides, the double bond in the carbohydrate decreases the conformational flexibility: from 3 to 4 atoms in the sugar ring are nearly coplanar. In some other compounds (**V-X, XX-XXI** and **XXX-XXXI**), an extra ring attached to the sugar also causes limited flexibility. Nucleosides of tetrose (**XV-XVI** and **XXII-XXIII**) also have nearly planar glycone structures. Acyclic unsaturated

Table 3. Interaction of neplanocin A, its analogues and some other compounds with different enzymes

Compound	Substrate for enzymes	inhibitor for enzymes
XXVI (NepA)	humans: Ado-kinase[65], adenylatekinase[65], nucleoside diphosphate kinase[65], RNA polymerases[67], catechol-O-methyltransferases[67], Ado-deaminases[65]	Ado-Hcy-hydrolase[68], neurotransmitter methyltransferases,[66] protein methyltransferases,[66]- nucleic acid methyltransferases,[66] lipoprotein methyltransferases[66]
XL, XLI, XLIII, XLIV		Ado-Hcy hydrolase[66]
XLII	calf intestine Ado-deaminase[76] human UTP-aminotransferase (CTP synthetase)[80],	
XLIII, XLIV,	human nucleoside kinase, nucleotide kinase, nucleoside diphosphate[79] kinase[80], RNA polymerase[80]	ribonucleoside diphosphate XLV reductase[81] and uridine kinase[79]
XLVI		uridine kinase[79]

nucleosides, such as XXV and XL-XLIV, also contain a less flexible hydrocarbon chain fragment due to the presence of a double bond.

CONCLUSION

In summarizing all these data, we can draw some general conclusions.

1. A large group of enzymes that participate in the metabolism of nucleic acid components recognize the conformationally restricted modified nucleosides or nucleotides. These enzymes form complexes with these analogues of the nucleic acid. Among these we have dealt with the following enzymes: nucleoside kinases, nucleotide kinases, pyrophosphoryltransferases, more then 15 DNA polymerases of different structure and origin, RNA polymerases, adenosine and cytidine deaminases,

nucleoside phosphorylases, UTP transferase, AdoMet synthetases, AdoMet decarboxylase, polyamine synthetase, Ado-Hcy hydrolase, group of methyltransferases and some others.

2. Many modified nucleosides and nucleotides of different structural features are able to bind to the active sites of the above-mentioned enzymes. Such nucleoside molecules have one general property: they have a conformationally restricted sugar residue, which are more planar than those in the natural nucleosides or their 5'-phosphates.

3. The sugar residue may, therefore, be able to guide the nucleobase to the right orientation with respect to reactive groups for binding of the nucleoside at the active center of the enzymes. It is the 5'-hydroxyl group of nucleosides that is involved in the reactions catalyzed by nucleoside and deoxynucleoside kinases, 5'-phosphotransferases, and possibly some other phosphorylation enzymes. For nucleotide kinases, 5'-phosphoryl-1-pyrophosphoryltransferases and DNA and RNA polymerases, the corresponding phosphate residues are the site of reaction. Sometimes special recognition group may be required, when the sugar residue does not take part in the transformation reaction. For example, the site of reaction for nucleoside deaminases is not the sugar moiety, although the importance of the sugar structure in the deaminase reaction is well known.[81,82] It can be argued that some hydroxy groups in the sugar can be replaced by hydrogen or other substituents without perturbing conformational properties of the sugar. Of course, 3'-hydroxyl can interact with a functional group in active center of enzyme and in this way increase the affinity of nucleoside or nucleotide substrates for the enzyme. There are more complicated cases, such as the elongation of DNA and RNA: the 3'-hydroxyl group of nucleotide residue, after being incorporated into the 3'-terminus of DNA and RNA chain, takes part in the subsequent step for chain elongation. These reactions, however, are beyond the scope of our discussion, since they belong to a different type of enzyme-substrate interactions.

4. Many enzymes which catalyze metabolic pathways of nucleic acid components possess, as a whole, similar conformational motif of the active center.

5. Because substrate analogues with conformationally restricted sugar can change their conformation only to a limited degree without great energy cost, their

investigation opens pu the possibility to construct the recognition site of enzyme active sites.

As stated in the Introduction, the purpose of this account is to present a simple hypothesis for the various types of enzyme reactions in which nucleosides and nucleotides participate, and to stress the interrelationship which exist among them when viewed from this hypothesis. The readers should bear in mind that this hypothesis cannot explain the differences between active and inactive compounds in some enzymatic processes, but it opens an opportunity to make conformational models of the active sites of some enzymes and in this way to help predicting properties of new model substrates or inhibitors of these enzymes. Determination of this unique motif of the active center for these groups of enzymes in quantitative physico-chemical terms will open a new area of research.

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