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SOME RECENT FINDINGS IN THE BIOTECHNOLOGY OF BIOLOGICALLY IMPORTANT NUCLEOSIDES

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Some recent findings in the biotechnology of biologically important nucleosides will be discussed, *viz.*, (i) a new strategy of the cascade one-pot transformation of D-pentoses into nucleosides based on the extension and deepening of the knowledge of the mechanism of functioning of the ribokinase, phosphopentomutase, and uridine, thymidine and purine nucleoside (PNP) phosphorylases, and the role of different factors (structural, electronic, stereochemical) in the glycoside bond formation, (*ii*) the modern chemistries of the chemo-enzymatic syntheses of nucleosides, (*iii*) the transglycosylation reaction using natural and sugar modified nucleosides as donors of carbohydrate residues and heterocyclic bases as acceptors catalyzed by nucleoside phosphorylases (NP).

Key words: nucleosides, bio-mimetic synthesis, chemo-enzymatic synthesis, enzymes of nucleic acid metabolism.

Nucleosides embrace a large family of natural and chemically modified analogues of great structural diversity and a broad spectrum of biological activity. Analogues of natural nucleosides as well as nucleoside antibiotics belong to the most important classes of antiviral drugs, and they are extensively used in the treatment of a variety of cancers. Base and sugar modified nucleosides are very valuable constituents of artificial oligonucleotides of medicinal potential making these oligomers more stable in biological fluids and improving their targeting proper-ties. The chemistry of many antiviral and anticancer drugs, as well as building blocks for oligonucleotide synthesis remains a challenging problem resulting in a high price of the desired compounds preventing them from extensive therapeutic application and use in oligonucleotide business (for a recent reviews, see [1–3].

Analysis of the state of the art of chemo-enzymatic synthesis of nucleosides led us to conclusion that the chemo-enzymatic methodology demonstrates a number of advantages over the chemical methods of nucleoside synthesis, *viz.*, high total yield of desired products, simplicity of work-up of reaction mixtures and isolation of products, conform to the principles of «green chemistry» to a greater extent *vs.* the fine organic synthesis [1–3]. Up to the present, a vast majority of the modified nucleosides have been synthesized by chemical methods. Despite the impressive progress achieved in the development of chemical methods, production of many antiviral and anticancer drugs, as well as other biologically active compounds remains a challenge. This leads to high drug costs and, therefore, prevents extensive biological trials and studies, as well as a wide therapeutic application. The need for the development of new strategies became apparent in the late 1970s.

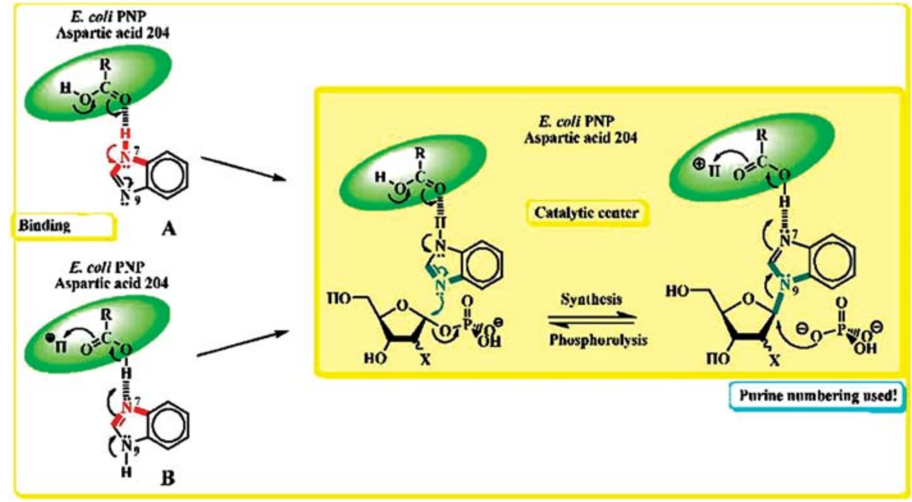
The chemo-enzymatic (biotechnological) strategies are currently displacing multi-stage chemical processes, and this allows performing the key transformations with high selectivity and *regio*- and *stereo*-specificity. Considerable progress in the production of biologically important analogs of natural nucleosides has been achieved through the rational combination of chemical and biochemical transformations. Use of recombinant nucleoside phosphorylases (NPs) and *N*-deoxyribosyl transferases (NDTs) as biocatalysts for the synthesis of natural nucleosides and their modified analogs is of considerable importance

for the creation of modern technological processes. Noteworthy that both groups of enzymes complement one another and allow finding out a straightforward route to the desired compound. The use of the chemo-enzymatic methods allows undoubtedly improve the price-quality ratio in the production of many medical drugs.

The possible areas of application of nucleoside phosphorylases for the synthesis of nucleosides, as well as the limitations of this methodology, have been investigated in detail; however, several very interesting enzymatic synthetic reactions deserve special attention, because they are crucial for understanding the mechanism of synthetic reactions catalyzed by these enzymes and may expand the scope of their practical use. It is well documented that the N^7-atom of purines plays a very important role in the phosphorolytic cleavage of the glycosyl bond of purine nucleosides and, it seems, in the reversed synthetic reaction catalyzed by *E. coli* purine nucleoside phosphorylase (PNP; product of *deoD* gene; EC 2.4.2.1) as well, even though the mechanism of this reaction has not been adequately studied. The finding that 3-deazapurines and 1-deaza-, 3-deaza- and 1,3-dideazapurines (benzimidazoles, including fluoro-, chloro- and bromo-substituted) are good substrates for PNP allows to suggest a key role for two nitrogen atoms of the imidazole ring in the synthetic reaction (for a discussion, see [1]). Namely, one of them is involved in the binding of the heterocyclic base in the enzyme's active site that may lead to an increase of the nucleophilicity of the second nitrogen atom. This facilitates, in turn, an attack by this atom on the electrophilic C1 carbon atom of α-D-pentofuranose-1-phosphate and eventually results in the formation of a glycosidic bond (Scheme 1) [2].

The mechanism of the synthetic reaction catalyzed by nucleoside phosphorylases in general and PNP in particular remains unclear. The participation of two nitrogen atoms of benzimidazole in this reaction seems to be obvious albeit there are two modes **A** and **B** of initial binding of the substrate. The Check authors studied substrate properties of a number of purine heterocyclic bases and their aza- and deaza-analogues using partially purified *E. coli* PNP and gel-entrapped cells of an auxotrophic thymine-dependent strain of *E. coli* as a biocatalyst for the transfer of the 2′-deoxy-D-ribofuranosyl moiety of 2′-deoxyuridine to bases. The reactions proceeded *regio*- and *stereo*-specifically affording purine nucleosides as well as 8-aza-2′-deoxyadenosine and 8-aza-2′-deoxyguanosine but no substrate activity of 7-deazapurines was observed. It was thus concluded that the presence of the nitrogen-7 of purines and their isosteric analogues is a prerequisite for the reaction [4]. However, there are several exceptions, *viz.*, 5-aza-7-deazaguanine **(1)**, 5-aza-7-deazaisoguanine **(2)** and *N*-(1,3,4-thiadiazol-2-yl)-cyanamide **(3)** are substrates for bacterial and mammalian purine nucleoside phosphorylases (PNP's) (Scheme 2) [2].

These data imply that the mechanism of binding and activation of substrate in the catalytic center of *E. coli* PNP is not uniform and prompted us to gain insight into the functioning of the enzyme and to search for new substrates and inhibitors.



Scheme 1

Scheme 2

Results and Discussion

The main line of the present study based on the suggested by us new strategy of nucleoside synthesis consisting in the cascade transformation of pentoses into nucleosides through intermediate consecutive formation of a pentofuranose-5-phosphate and α-D-pentofuranose-1-phosphate (PF-α1P) catalyzed by recombinant *E. coli* ribokinase (RK), phosphopentomutase (PPM) and nucleoside (uridine, thymidine and purine nucleoside) phosphorylases (Scheme 3) [5]. Before an idea of the cascade transformation — D(L)-pentose + base → nucleoside — was proved, the chemical synthesis of PF-α1P was considered as an important supplementary approach to the preparation of sugar and base modified nucleosides for biological and medicinal application. The chemo-enzymatic nucleoside synthesis consisting of the chemical preparation of PF-α1P followed by an enzymatic condensation with heterocyclic bases seemed to be more versatile and rather attractive.

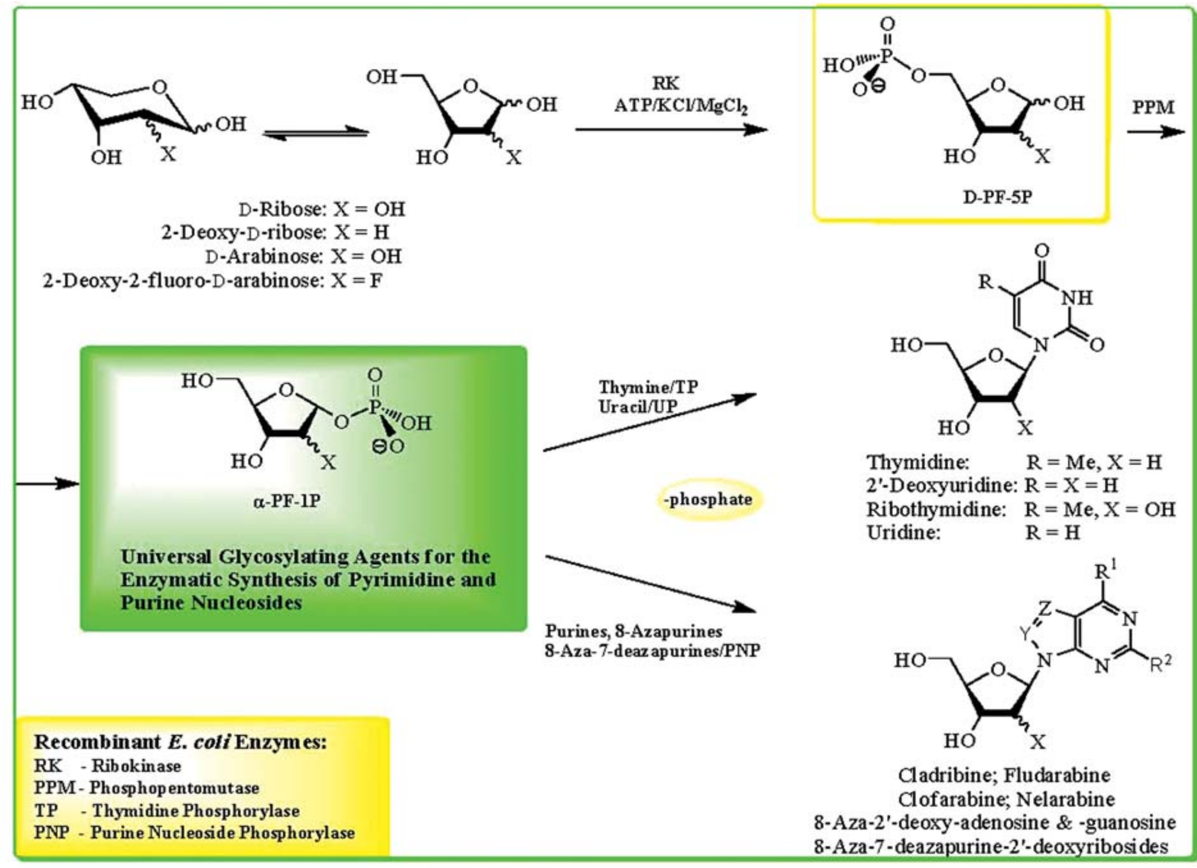
Preparation of recombinant* E. coli *ribokinase (RK), phosphopentomutase (PPM) and nucleoside phosphorylases (NP's) on the practical level is of utmost importance for the project and this task was realized and all these enzymes were obtained as they spend at work [2, 3, 5–7]. Taking into account the aims of the present study, the preparations of the aforementioned enzymes have been obtained in different forms [lyophilized powders of high enzymatic (>90% after 3-5 years storage at +4 °C) stability], solution of a protein in diverse buffers] and different levels of purity (carefully purified for biochemical studies; sufficient purity for biotechnological studies).

A possibility of cascade one-pot enzymatic transformation of D-ribose or 2-deoxy-D-ribose into nucleosides employing pure recombinant *E. coli* ribokinase (RK) [D-pentose → pentose-5-phosphate (D-PF-5P)], PPM [D-PF-5P → α-D-pentofuranose-1-phosphate (D-PF-α1P)], and nucleoside phosphorylases (NP's) (D-PF-α1P + heterobase → nucleoside) coupled with the appropriate pyrimidine or purine heterobases was demonstrated (Scheme 4) [7]. Preliminary results of a caskade transformation of D-pentoses into nucleosides pointed to a reliability to develop practical methods for the preparation of antileukemic drugs (*Cladribine*, *Fludarabine*, *Clofarabine*, *Nelarabine*) and a number of biologically important nucleosides.

We noted rather essential differences between the optimal reaction conditions for RK [5], PPM [7] and recombinant *E. coli* nucleoside phosphorylases [6]. Bearing this in mind, we have optimized the one-pot reaction conditions aiming at the finding out a compromised composition of the substrates allowing satisfactory function of the enzymes under investigation. It was found that D-ribose, 2-deoxy-D-ribose, D-arabinose and 2-deoxy-2-fluoro-D-arabinose are transformed into the respective nucleosides in good yields; D-xylose as well as some 2(3)-deoxyfluoro-D-pentofuranoses and L-pentofuranoses are not involved in the cascade transformation into nucleosides. This study is continued.

UP and **TP** - uridine and thymidine phosphorylase, respectively
PNP - purine nucleoside phosphorylase
RK - ribokinase
PPM - phosphopentomutase
Base - pyrimidine or purine heterocyclic base, as well as all bases that are substrates of UP, TP and PNP

Scheme 3



Scheme 4

Our studies unambiguously showed for the first time that 1,6-diphosphates of D-hexoses are not necessary for the transformation of 5-phosphates of D-ribose, 2-deoxy-D-ribose, D-arabinose and 2-deoxy-2-fluoro-D-arabinose into the corresponding 1-phosphates. This is one of the most important findings because it essentially simplifies the application of PPM as a biocatalyst within the one-pot cascade transformation of D-pentoses into nucleosides as well as for the transformation of chemically prepared 5-phosphates into intermediary 1-phosphates and then into nucleosides (Scheme 4) [7].

The strategy of cascade one-pot synthesis of nucleosides suggested by us [5,7] is of interest for development of practical methods for the preparation of biologically important nucleosides. It should be emphasized that this strategy allows preparing β-D-*ribo*-, 2-*deoxy*-β-D-*ribo*-, β-D-*arabino*- and 2-*deoxy*-2-*fluoro*-β-D-*arabino*-nucleosides of natural purine and pyrimidine (except for 2-*deoxy*-2-*fluoro*-β-D-*arabino*-nucleosides) bases, as well as base modified nucleosides. Indeed, R&D of this strategy led us to the simple and efficient preparation of antileukemic drugs 2-chloro-2β-deoxyadenosine (*Cladribine*) from 2-deoxy-D-ribose and 2-chloroadenine, 9-(β-D-arabinofuranosyl)-2-fluoroadenine (*Fludarabine*) (D-arabinose + 2-fluoroadenine), 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-2-chloroadenine (*Clofarabine*) (2-deoxy-2-fluoro-D-arabinose + 2-chloroadenine) and 2-amino-9-(β-D-arabinofuranosyl)-6-methoxypurine (*Nelarabine*) (D-arabinose + 2-amino-6-methoxypurine). Moreover, preliminary results of the synthesis of a number of biologically important nucleosides [*e. g.*, 2′-deoxyribosides of 8-azapurines and 8-aza-7-deazapurines (*vide infra*)] have validated this strategy [8].

Transfer of a pentofuranosyl moiety of commercially available nucleosides or prepared by chemical methods to purine or pyrimidine bases catalyzed by nucleoside phosphorylases (NPs) or *N*-deoxyribosyltransferases (DRTs) («transglycosylation reaction») was demonstrated to be a very efficient methodology for the synthesis of a plenty of analogues of natural nucleosides of biological and medicinal importance (for recent reviews, see, *e. g.*, [1–3]). The bacterial nucleoside phosphorylases [purine (PNP), thymidine (TP) and uridine (UP)] reversibly catalyze (*i*) the phospho-

rolysis of nucleosides with an intermediary formation of α-D-pentofuranose-1-phosphate (PF-α1P), and (*ii*) the synthesis of new nucleosides in the presence of external heterocyclic bases. As distinct from nucleoside phosphorylases, DRTs catalyze the direct transfer of the 2′-deoxyribofuranosyl moiety of donors without intermediary formation of 2-deoxyribofuranose-1-phosphate. From the viewpoint of practical synthesis of sugar and base modified nucleosides, NP and DRT complement each other, but the latter have a strict specificity towards substrates thereby limiting their application.

Base halogenated nucleosides of benzimidazole attract an attention of researchers since the pioneering studies by I. Tamm and his co-workers initiated in the early 1950s of the last century (for a review, see [9]). However, the most important findings from the viewpoint of the biochemical mechanism of antiviral activity of this class modified nucleosides [10] as well as their possible practical application [11] have been published during last two decades (reviewed in [12,13]).

It was earlier shown that benzimidazole (BI) and its derivatives with substituents in the benzene ring are good substrates of *E. coli* PNP in the transglycosylation reaction [1, 14–17]. In the present project, we studied 5,6-difluorobenzimidazole and its derivatives, one fluorine atom of which is replaced with methoxy, ethoxy, isopropoxy, 4-morpholino and *N*-methylpiperazino groups, as acceptors of the D-ribofuranose and 2-deoxy-D-ribofuranose residues in the transglycosylation reaction employing uridne (Urd) and thymidine (Thd) [2′-deoxyuridine (dUrd)], respectively, as the pentofuranose donors and recombinant *E. coli* UP, TP and PNP nucleoside phosphorylases as biocatalysts [18].

The reaction transglycosylation was employed for the synthesis of the β-D-ribo- and 2-deoxy-β-D-ribonucleosides **4–15**. The reaction conditions have been optimized depending on the kind of a ribofuranose donor, the donor/base ratio, and quantity of the recombinant *E. coli* enzymes and temperature of the reaction. The use of readily available natural purine ribonucleosides as donors of the ribofuranose residue in the transglycosylation reaction of BI allows employing PNP as the sole biocatalyst. On the contrary, the use of uridine as a donor of the ribofuranose residue requires two nucleoside phosphorylases for transglycosylation reactions, *viz*., the recombinant *E. coli* uridine phosphorylase (UP) for the intermediary formation of α-D-ribofuranose-1-phosphate (D-Rib-α1P) that is accepted by *E. coli* PNP for the synthesis of the BI ribosides. The efficiency of both types of donors was tested in the transglycosylation of 5,6-difluorobenzimidazole and it was found that the use of uridine and two nucleoside phosphorylases is preferable in terms of yield of the desired nucleosides.

It was found that the 3:1 to 10:1 molar donor/base ratio and the use of 40 units of uridine phosphorylase (UP) per 1 mmol of uridine (60–160 UP units for 2′-deoxyuridine]) and 155–400 units of PNP are necessary to obtain the ribosides **4–15** in good yields calculated for isolated products (Table 1). Reactions were conducted at 52 °C in the *K,Na*-phosphate buffer (5–20 mM; pH 7.0) monitoring the formation of the products by HPLC, the conversion of base into nucleoside(s) was 98.5%. It is noteworthy that the synthesis of 2′-deoxyribosides was completed in 1–3 h, whereas the *trans*-ribosylation proceeded much more slowly and 22–28 h required achieving high yields of the reaction products. Similar trend was earlier observed in the case of the *ribo* and *2′-deoxyribo* nucleoside syntheses using the whole *E. coli* cells as biocatalyst (Scheme 5) [18].

Scheme 5

Table 1. **The synthesis of benzimidazole nucleosides**

Compd	Pfr [1)]	R	Yield for isolated product (%)	Ratio of isomers	
				a	b
4	Rib	-F	77	–	
5	dRib		68	–	
6	Rib	-OMe	54	95	5
7	dRib		51	41	59
8	Rib	-OEt	98	89	11
9	dRib		85	70	30
10	Rib	-O*i*Pr	75	100	–
11	dRib		65	96	4
12	Rib	–N(morpholino)O	75	100	–
13	dRib		80	100	–
14	Rib	–N(piperazino)N-Me	79	100	–
15	dRib		68	100	–

Pfr = β-D-Pentofuranosyl (Rib — β-D-ribofuranosyl; dRib — 2′-deoxy-β-D-ribofuranosyl).

Regio-isomeric structure of all isolated nucleosides was proved by scrupulous analysis of the ^{1}H and ^{13}C NMR spectral data (incl. [^{1}H,^{1}H] & [^{1}H,^{13}C] 2D: COSY, HSQC, HMBC and NOE spectra).The predominant (OMe and OEt) or exclusive (O*i*Pr, 4-morpholino and *N*-methylpiperazino) formation of the 5-substituted 6-fluoro-1-(β-D-ribofuranosyl)-benzimidazoles was observed. The formation of the *regio*-isomeric 5-fluoro-1-(2-deoxy-β-D-ribofuranosyl)-6-methoxy(ethoxy, *i*-propoxy)benzimidazoles was observed in the trans-2-deoxyribosylation reaction of the corresponding bases. The predominant or exclusive formation of the *regio*-isomeric N^{1}-nucleosides with bulky 5-substituents of 6-fluorobenzimidazole points to a large hydrophobic pocket in the *E. coli* PNP active site that can accommodate these groups.

During recent years, the use of D-pentofuranose-1-phosphates (PF-1Ps) as substrates of an enzymatic synthesis of nucleosides attracts much attention [1–3]. It should be stressed that the enzymatic and chemical syntheses of D-ribofuranose-1-phosphate and its 2-deoxy counterpart have rather reach prehistory. However, only recently a few interesting reports from the point of view of the possible practical application have been published. There are two lines of investigation in this area, *viz*., (*i*) biochemical (microbial, enzymatic) *retro*-synthesis of 2′-deoxyribonucleosides employing the triose phosphate isomerase (TRI) and 2-deoxy-D-ribose-5-phosphate aldolase (DERA) enzymes and (*ii*) chemical synthesis of D-pentofuranose-1-phosphates (PF-1Ps) followed by the enzymatic condensation with heterocyclic bases.

Within this line of investigation, the laborious preparation of the α-D-pentofuranose-1-phosphates (PF-α1Ps) is a serious bottleneck of this approach. However, despite rather complex synthesis of PF-α1Ps this chemo-enzymatic methodology for the synthesis of biologically valuable nucleosides represents an advisable alternative to an enzymatic transfer of sugar fragment of nucleoside to heterocyclic base («transglycosylation reaction») as well as to the microbial (enzymatic) *retro*-synthesis [1–3].

Scrutiny of the chemical methods for the preparation of pento(hexo)furanose-1-phosphates as well as different methods of an activation of the anomeric carbon atom shows that the most of them are laborious and low-yielding. As might be expected, the formation of anomeric mixtures was usually observed, and only the tedious crystallization-induced asymmetric transformation leads to the predominant formation of the desired dRF-α1P (reviewed in [1–3]). From the standpoint of simplicity, method suggested by MacDonald [19] for the synthesis of pyranose-1-phosphates seems to be the most efficient one and prompted us to apply it for the synthesis of α-D-arabinofuranose-1-phosphate (**19**; Ara^{F}-α1P) [20]. We focused our studies on the development of practical chemical synthesis of PF-1Ps and selected D-arabinose as a starting pentose bearing in mind that a plenty of

purine and pyrimidine β-D-arabinofuranosides manifest antiviral and anticancer activities.

It is remarkable that Ara^F-α1P synthesized by *Wright & Khorana* was found to be inactive as a substrate for the pyrimidine deoxyriboside phosphorylase of *E. coli* (thymidine phosphorylase, TP) and for the purine nucleoside phosphorylase (PNP) of fish muscle [21]. These data taken together prompted us to synthesize Ara^F-α1P and to investigate its substrate properties for recombinant PNP of *E. coli*.

Treatment of the peracetyl derivative of D-arabinose **16** with anhydrous phosphoric acid under MacDonald' conditions followed by work-up gave rise to the formation of an amorphous powder or viscous oil consisting of α-D-arabinofuranose-1-phosphate (**19**; Ara^F-α1P) and β-D-arabinopyranose-1-phosphate (**20**; Ara^P-β1P) (*ca*. 50%, combined; the **19:20** ratio was from 1.5:8 to 1:2, according to ^{1}H NMR). The structure of both phosphates was proved by (*i*) the scrupulous analysis of the ^{1}H and ^{13}C NMR spectra as well as [^{1}H,^{1}H] and [^{1}H,^{13}C] 2D spectra of the mixtures of different anomer ratio, (*ii*) comparison with the ^{1}H and ^{13}C NMR data for the related 1-phosphates, and (*iii*) the comparative analysis of the *ab initio* calculations of 1-phosphates of selected α-D-pentofuranoses and two conformers, $_1C^4$ and $^1C_4$, of β-D-arabinopyranose (Table 2).

In consent with experimental results, the *ab initio* calculations point to a higher stability of both possible conformers of β-D-arabinopyranose-1-phosphate, *viz*., $_1C^4$ and $^1C_4$, *vs* α-D-arabinofuanose-1-phosphate [$\Delta E = E$(AP-β1P; $_4C^1$) — E(AP-β1P; $^4C_1$) = −11.7 kcal/mol; [$\Delta E = E$(AP-β1P;$_4C^1$) — E(AF-α1P; O4-*exo*) = −26.9 kcal/mol] (Table 2). It was previously shown that the ratio of α- and β-anomers strongly depends on the reaction conditions implying the α/β-anomerization to the thermodynamically more stable anomer and the possibility of furanose/pyranose isomerization during the treatment of peracyl sugars with anhydrous phosphoric acid cannot be excluded. *In toto*, it appears to be rather likely the formation of β-D-arabinopyranose-1-phosphate along with the desired α-D-arabinofuranose-1-phosphate using peracetyl D-arabinofuranose as the starting compound. In this context, it is noteworthy that the *MacDonald* method was up to present study successfully employed for the synthesis pyranose-1-phosphates [19]. To escape or diminish the formation of the pyranose-1-phosphates, we focused our further studies on the preparation of new starting sugars, primary hydroxyl group of which would be protected with an acid resistant function. However, methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (**17**) was stable under MacDonald's reaction conditions and was recovered unchanged from the reaction mixture; on the contrary, 1-O-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose (**18**) showed good reactivity, but gave a mixture of the 1-phosphates **19** and **20** similar to that obtained from the peracetyl derivative of D-arabinose. In toto,

The mixture of isomeric phosphates Ara^F-α1P and Ara^P-β1P of different ratios was tested in the reaction with 2-fluoroadenine and 2-amino-6-methoxypurine catalyzed by the recombinant *E. coli* purine nucleoside phosphorylase (PNP). It was found that the pyranose phosphate Ara^P-β1P did not interfere with the reaction of the furanose phosphate Ara^F-α1P with purine bases. Moreover, the rate of formation of 9-(β-D-arabinofuranosyl)-2-fluoroadenine (*Fludarabine*) under optimum conditions [water solution (pH = 7.0); 55 °C,

Table 2. **The *ab initio* geometry optimization of α(β)-D-pentofuranose(pyranose)-1-phosphates (as mono sodium salts) (HyperChem, 8.1; *in vacuo*, basis set; medium 6-31G*)**

Compound (1-Phosphate)	Positive partial charge at the C1 carbon atom	Total (binding) energy kcal/mol	Conformation of the pentofuranose(pyranose) ring
Ribo (RF- *α* 1P)	0.425	−808 850.3	C1-exo
2-Deoxyribo (dRF- *α* 1P)	0.454	−762 140.7	C3-endo
Arabino (AF- *α* 1P) [a)]	0.464	−808 841.6	O4-exo
β-D-*Arabinopyranose*	0.410	−808 868.5	$_4C^1$(more stable)
(AP-β1P)	0.451	−808 856.8	$^4C_1$(less stable)

[a)]α-D-arabinofuranose-1-phosphate is thermodynamically less stable *vs* both conformers of β-D-arabinopyranose-1-phosphates, *viz*., $_4C^1$ and $^4C_1$; note that among the two pyranose conformers the former is more stable than the latter. Colored data are for isomeric compounds with analogous elemental composition.

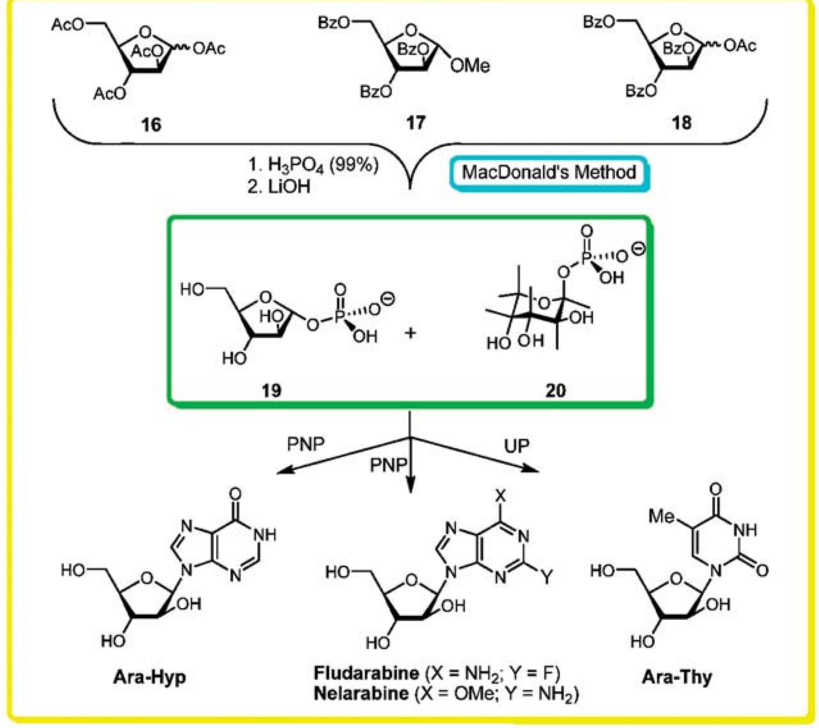
1–3 h] was found to be similar to that of the PNP catalyzed condensation of α-D-ribofuranose-1-phosphate with 2-fluoroadenine. Reaction of furanose phosphate Ara^{F}-α1P with 2-fluoroadenine is shifted towards the formation of *Fludarabine* and *ca*. 5% of the initial base remained after 3 h in the reaction mixture, keeping of which at 14 °C for 24 h resulted in crystallization of *Fludarabine* in 77% yield (Scheme 6) [20].

As distinct from 2-fluoroadenine, the enzymatic reaction of 2-amino-6-methoxypurine with phosphate Ara-α1P reached equilibrium at *ca*. equimolar ratio of a base and its nucleoside, 2-amino-9-(β-D-arabinofuranosyl)-6-methoxypurine (*Nelarabine*). Notably that the reaction transglycosylation of 2-amino-6-methoxypurine using 1-(β-D-arabinofuranosyl)uracil as a donor of the arabinofuranose residue and the *E. coli* uridine and purine nucleoside phosphorylases as biocatalysts gave rise to the preparation of *Nelarabine* in 53% yield [22]. Unexpectedly, 2-amino-6-methoxypurine revealed lower substrate activity compared to that of 2-fluoroadenine, whereas the respective nucleoside *Nelarabine* showed higher substrate activity for PNP *vs Fludarabine*. As a consequence, the reaction reached equilibrium after 36 h at *ca*. equimolar concentration of base and its nucleoside. It is noteworthy that very similar results were observed in the case of the *trans*-ribosylation [uridine as a donor of the ribofuranose residue; 23 °C; 5 mM KPB (pH 7.0)] and the *trans*-arabinosylation [1-(β-D-arabinofuranosyl)-uracil as a donor of the arabinofuranose residue; 45 °C; 5 mM KPB (pH 7.0)] of 2-amino-6-methoxypurine employing UP and PNP as biocatalysts [20].

Unexpectedly, the enzymatic reaction of hypoxanthine and a mixture of phosphates **19** and **20** (*ca*. 1:2) in the presence of PNP (172 units) in water solution (55 °C) proceeded very slowly. After 7 days, in the reaction mixture remained *ca*. 10% of the starting base (HPLC) and 9-(β-D-arabinofuranosyl)-hypoxanthine (Ara-Hyp) was isolated in 80% yield after standard work-up and chromatography.

The synthesis of 1-(β-D-arabinofuranosyl)thymine (ara-Thy) from thymine and the phosphate **19** (as a *ca*. 1:2 mixture of phosphates **19** and **20**) in water solution (55 °C) in the presence of the recombinant *E. coli* thymidine (TP) and uridine (UP) phosphorylases was studied. As expected (*cf*. [21]), TP did not catalyze the formation of the nucleoside. The formation of ara-Thy in the presence of *E. coli* UP (144 units) proceeded smoothly and *ca*. 70% of the base was transformed into ara-Thy after 7 days that was isolated in 61% yield [20].

In continuation of these studies, we investigated the chemical synthesis of 2-deoxy-2-fluoro-α-D-arabinofuranose-1-phosphate (^{2F}Ara-α1P) and its substrate properties for



Scheme 6

the recombinant *E. coli* nucleoside phosphorylases, as well as the cascade transformation of 2-deoxy-2-fluoro-D-arabinose, D-arabinose, D-xylose and D-ribose into the corresponding β-D-pentofuranosides of 2-chloroadenine. The modified MacDonald method was employed for the synthesis of ^{2F}Ara-α1P and its use as a universal glycosylating agent for the nucleoside synthesis was studied in comparison with α-Ara-1P. It was found that the phosphate α-^{2F}Ara-1P, like α-Ara-1P, is good substrate of the recombinant *E. coli* purine nucleoside phosphorylase (PNP) and can be used for the synthesis of a number of purine nucleosides (*e.g.*, *Clofarabine* and related nucleosides of 2,6-diaminopurine and hypoxanthine) as well as base modified derivatives [*e.g.*, 5-aza-7-deaza-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine]. Unexpectedly, the phosphate α-^{2F}Ara-1P, unlike α-Ara-1P, showed no substrate activity for the recombinant *E. coli* uridine phosphorylase (UP); both phosphates devoid substrate activity towards the recombinant *E. coli* thymidine phosphorylase (TP) (for a preliminary report, see [23]).

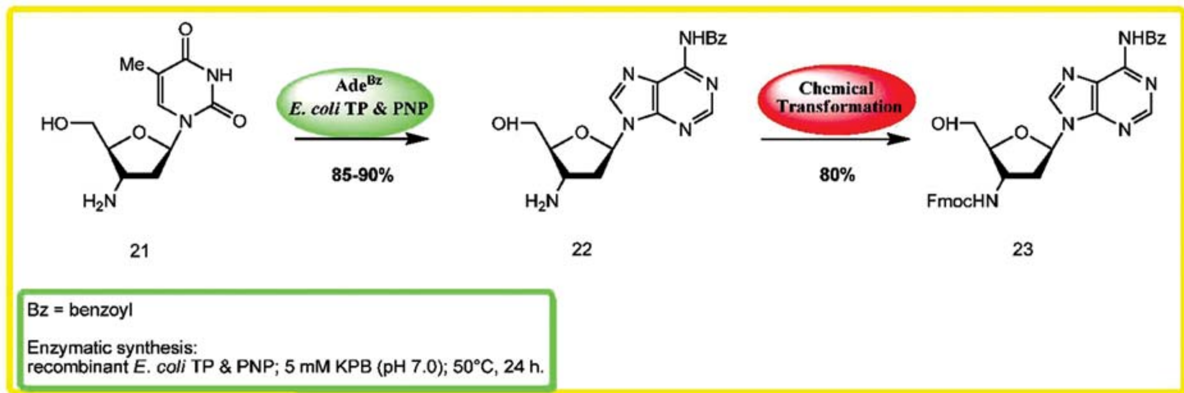
Recently we have described an enzymatic synthesis of nucleosides of N^6-benzoyladenine and N^2-acetylguanine using the respective acylated bases as acceptors of the pentofuranose residues and recombinant* E. coli *PNP as a biocatalyst [24]. Based on this finding, a new approach for the synthesis of orthogonally protected nucleosides was suggested and verified by the preparation of *N*^{6}-benzoyl-2′,3′-dideoxy-3′-Fmocaminoadenosine **(23)**. Commercially available 3′-amino-3′-deoxythymidine **(21)** and *N*^{6}-benzoyladenine were used as substrates of the enzymatic coupling catalyzed by *E. coli* PNP to give 3′-amino-2′,3′-dideoxy-*N*^{6}-benzoyladenosine **(22)** in high yield. Standard treatment of the latter with Fmoc-OSU yielded the desired nucleoside **23** with orthogonally protected amino groups of 3′-amino-2′,3′-dideoxyadenosine (Scheme 7) [24].

These studies were continued using *N*^{2}-acetyl-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanine **(24)** [25] as an acceptor in (*i*) the transglycosylation reaction employing thymidine **(25)** and its 3′-aminodeoxy derivative **(26)** as donors of the pentofuranose residues and recombinant *E. coli* TP and PNP as biocatalysts (Scheme 9, path *A*), and (*ii*) the synthesis of the *ribo*- and *arabino*-nucleosides **29** and **30** in the cascade one-pot transformation of the corresponding D-ribose **(27)** and D-arabinose **(28)** into the nucleosides in the presence of the recombinant *E. coli* RK, PPM and PNP (Scheme 9, path **B**).

The base **24** is poorly soluble in the phosphate buffer and a 3:2 (vol) mixture of *K*-phosphate buffer (20 mM; pH 7.1) and DMSO was, therefore, used in the studied reactions. It was found that the base **24** displays satisfactory substrate activity for PNP and the nucleosides **29** and **30** form in the reaction mixture in 64 and 42% (HPLC data), respectively, after 30 h at 50 °C (Scheme 8, *A*). Both individual nucleosides have been isolated by silica gel column chromatography in 50 and 34% yields, respectively. The use of the acceptor/donor ratio of 1.0:1.5 (mol) gave rise to the formation of the nucleosides **29** and **30** in 78 and 60% yields (HPLC), respectively, after 96 h at 50 °C [26].

A good substrate activity of the base **24** for PNP prompted us to test it in the one-pot synthesis of nucleosides from D-pentoses in the cascade transformation in the presence of RK, PPM and PNP (Scheme 8, *B*). It was found that the riboside **33** forms in the reaction mixture in 65% yield after incubation for 48 h at 48 °C. Under similar reaction conditions, the formation of the arabinoside **34** proceeds more slowly affording 10% of the product after 48 h.

The finding that base **24** with voluminous NPE group shows good substrate activity points to a large hydrophobic pocket in PNP



Scheme 7

Scheme 8

active site that can accommodate this group. The dimensions of this pocket are illustrated by the overlay of imidazole fragments of the geometry optimized structures of *N*[2]-acetyl-*O*[6]-[2-(4-nitrophenyl)ethyl]guanine and 5-morpholino-6-fluorobenzimidazole (*vide supra*) (Fig. 1).

Remarkably, the pocket in the E. coli PNP active site exceeds the dimension of purine base and allows suggesting a minimal contribution of the C6 amino/carbonyl groups and N1 atom to the substrate binding.

Nucleosides of 8-azapurines and 8-aza-7-deazapurines (purine numbering throughout) are applicable as drugs and tools in chemistry, chemical biology and molecular diagnostics (see, e.g., [27–31]). Chemical synthesis of these nucleoside shape mimics suffers from the formation of mixtures of regio-isomers and α/β-anomers in the case of the synthesis 2-deoxy-D-pentofuranosides and arabinosides. This makes it necessary to conduct time-consuming separation. As a result, the desired nucleosides are obtained in moderate or low yields (e.g., [29,31]).

We applied the enzyme catalyzed glycosylation to diversity of heterocyclic bases belonging to the classes of 8-azapurines (35 & 36) and 8-aza-7-deazapurines (37–40) using various donors of the 2′-deoxy-D-ribofuranosyl residue and recombinant E. coli PNP as a biocatalyst (transglycosylation reaction; Scheme 9; path *A*) and the enzymatic cascade transformation of 2-deoxy-D-ribose into the nucleosides (Scheme 10; path B) using recombinant E. coli RK, PPM and PNP (one-pot synthesis). The enzymatic synthesis of N9-2′-deoxy-β-D-ribonucleosides of a number of 8-azapurines (44, 45) and 8-aza-7-deazapurines (46–49) was studied (Scheme 9) [8].

At first, the substrate activity of bases 35-39 was tested in the transglycosylation reaction performed under standard conditions using 2′-deoxyguanosine (41) as a glycosyl donor (Scheme 10, *A*). All these bases are satisfactory substrates and the respective nucleosides were obtained in good yields. The structure of the nucleosides 44–48 was confirmed by 1H- and 13C-NMR spectroscopy data and UV spectra as well by comparison with already published spectral data. We have not observed the formation of the regio-isomeric nucleosides.

In the next series of experiments, we studied the one-pot synthesis of nucleosides 44–48 with 2-deoxy-D-ribose and heterocyclic bases 35-39 in the presence of recombinant RK, PPM and PNP (Scheme 10; path *B*). Under reaction conditions employed, the formation of 8-aza-2′-deoxyadenosine (44) and 8-aza-2′-deoxyguanosine (45) proceeded slowly affording the nucleosides in moderate yields. On the contrary, 8-aza-7-deazapurines 37–39 showed satisfactory substrate activity and the respective nucleosides 46–48 were formed in yields more than 50% after 20 h [8].

Scheme 9

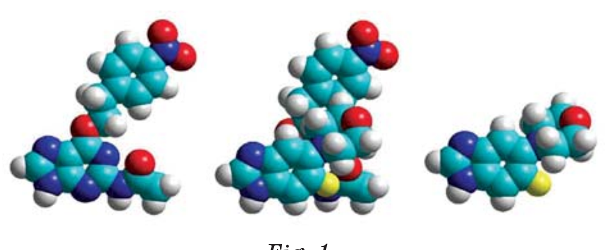


Fig. 1.

The regio-specific 2′-deoxy-D-ribosylation of 8-azaadenine (35) and 8-azaguanine (36) catalyzed by recombinant E. coli PNP is in line with published data [32]. On the contrary, glycosylation of anions of 8-azapurines with 2-deoxy-3,5-di-O-(4-toluoyl)-α-D-erythro-pentofuranosyl chloride gave a very complex mixture of regio-isomers and their α,β-anomers [28, 29].

From a chemical point of view, the formation of the glycosyl bond results from a nucleophilic attack of a nitrogen atom of base at the electrophilic C1-carbon atom of 2-deoxy-D-ribofuranose-1-phosphate (27). The regioselectivity of the enzymatic glycosylation is governed by the binding mode of base in the catalytic center of the enzyme enabling a nucleophilic attack of a nitrogen atom of the sp2 hybridized imino $-C=N^9-$ tautomer at the electrophilic center of co-substrate (Scheme 1). A similar type of binding of the substrate can be tolerated with a high probability for natural purine bases as well as for 8-azapurines 35 and 36 leading to the exclusive formation of N9-glycosides. However, the formation of nucleosides 46–48 was rather unexpected in the light of the earlier data pointing to the crucial importance of the nitrogen-7 of purines and their isosteric analogues in the synthetic reaction catalyzed by E. coli PNP [2, 3, 32].

Detailed analysis of the mechanism of the phosphorolysis of purine nucleosides by *E. coli* PNP showed that the base binding site is formed mainly by Asp204 and to some extent by Phe159 [33]. Taking into account that this is an equilibrium reaction, one can expect that the same amino acid residues make the main contribution in binding of substrate in the synthetic reaction. The Asp204 interacts with the nitrogen-7 and likely the C6 substituents of natural purine bases giving rise, in all likelihood, to the proper base orientation and to enhancement of the nucleophilic properties of the nitrogen-9, i.e., activation of the substrate. It is, therefore, surprising that replacement of the nitrogen-7 with CH group did not abolish the substrate activity of bases 37–39 pointing to the rather efficient contribution of the Asp204-C6 substituent interaction in the correct binding and activation [1]. To prove the role of such interaction, we investigated the substrate properties of base 40, which has no groups (NH_2, carbonyl, OH or

OR) that could imitate an interaction of natural purine substrates with Asp204. It was surprising to found that base 40 still retains moderate substrate activity despite the absence of any interactions with Asp204 and the extremely low solubility.

Analysis of the crystal structure of the ternary complex of hexameric *E. coli* PNP with Formycins A and B showed that the Ser90 is involved in binding of the bases [33a,b]. Moreover, crystallographic data for the adenine binding to the active site of *E. coli* PNP clearly showed a close proximity of Ser90·O^{γ} to the carbon-8 of the base [33c,d]. These data together with the aforementioned considerations suggest the possible explanation for the good substrate properties of bases 46–48 and moderate activity of base 49, viz., Ser90·O^{γ} is hydrogen bonded to nitrogen-8 (purine numbering) of the bases giving rise to the acceptable base orientation in the catalytic site of E. coli PNP followed by activation of the nitrogen-9 in productive complex 27 (Fig. 2). Contribution of Phe159 of E. coli PNP in both processes, binding and activation, appears to be analogous to that of the natural bases. Thus, in the case of 8-aza-7-deaza purine analogs Ser90 residue of the catalytic site of E. coli PNP takes effect of Asp204 in the case of natural purine substrates.

The spatial tautomeric structures of base 40 in complex with Ser90·O^{γ} have been analyzed by the restricted Hartree-Fock (RHF) ab initio method using basis set of 6-31** FIREFLY QC package, which is partially based on the GAMESS (US) source code. The files of MOPAC format containing Z-matrix of internal coordinates obtained by the PM3 geometry optimization was used as starting approximation for the ab initio calculations. The following main dimensions were obtained for the respective structure E. coli PNP/base 40 — Ser90-O^{γ}–H···N^8 0.97 & 1.86 E; Ser90-O^{γ}···H–N^9 2.39 & 0.99 E; =N^8–N^9(H)- 1.39 E; 27 — Ser90-O^{γ}···H–N^8 2.37 & 0.99 E; Ser90-O^{γ}–H···N^9 0.96 & 1.83 E; –N^8(H)–N^9 = 1.37 E; these data are in fair agreement with strong hydrogen bonding of the base enabling the correct binding followed by activation [8].

To prove this suggestion, the Ser90Ala mutant of *E. coli* PNP was prepared and its catalytic activity in the synthesis of purine and 8-aza-7-deazapurine nucleosides studied.

The transribosylation of hypoxanthine and 8-aza-7-deazahypoxanthine (allopurinol) using uridine and the recombinant *E. coli* uridine phosphorylase for the generation of

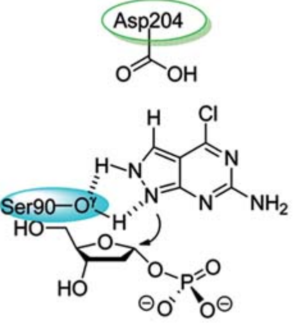


Fig. 2. **Schematic presentation of productive *E. coli* PNP/base 40 complex**

intermediary α-D-ribofuranose-1-phosphate revealed the differences in rates of the formation of inosine and allopurinol riboside (AR) along with high yields of both nucleosides. In the presence of the native E. coli PNP, the formation of (i) inosine reached 90% after ca. 30 min and after next 30 min established an equilibrium at ca. 2:8 base — nucleoside ratio in the reaction mixture, and (ii) 8-aza-7-deaza-9-(β-D-ribofuranosyl)purine (AR) proceeded somewhat slowly achieving a 95% conversion of allopurinol to its N^9-riboside 3a after 24 h, witnessing to non-critical role of the N^7-nitrogen atom in the binding and activation of heterocyclic substrate in the synthesis of nucleosides catalyzed by the native *E. coli* PNP. Replacement of the native PNP with the mutated enzyme in the synthetic reactions resulted in (i) the slowing down of the rate of the inosine formation (35% yield of inosine after 1 h; an equilibrated ca. 2:8 mixture of base — nucleoside after 24 h), and (ii) dramatic reduction of the rate of the AR formation attaining ca. 20% yield after 48 h. These data allow implying the moderate contribution of the Ser90 residue of the catalytic center of the native E. coli PNP in the binding of α-D-Rib-1P. Dramatic reduction of the rate of the allopurinol riboside formation points to an unique importance of Ser90 residue of the native *E. coli* PNP in the binding and activation of allopurinol as well as other 8-aza-7-deazapurines [especially 2-amino-8-aza-6-chloro-7-deazapurine (40)] in the enzymatic synthesis of their nucleosides (for a preliminary report, see [34].

Thus, the pure recombinant E. coli ribokinase (RK), phosphopentomutase (PPM) and nucleoside phosphorylases [uridine (UP), thymidine (TP) and purine nucleoside (PNP)] were prepared on a multigram level [5–7].

A new strategy for the synthesis of nucleosides consisting in one-pot enzymatictransformation of D-ribose, 2-deoxy-D-ribose, D-arabinose and 2-deoxy-2-fluoro-D-arabinose in the presence of heterocyclic bases into the pyrimidine and purine nucleosides was suggested and validated. It consists in consecutive transformation of D-pentoses into nucleosides under the action of recombinant E. coli ribokinase (RK) [D-pentose pentose-5-phosphate (D-PF-5P)], PPM [D-PF-5P α-D-pentofuranose-1-phosphate (D-PF-α1P)], and nucleoside phosphorylases (NPs) (D-PF-α1P + heterobase nucleoside). It was unambiguously shown for the first time that 1,6-diphosphates of D-hexoses are not necessary for the transformation of 5-phosphates of D-pentoses into the corresponding 1-phosphates. Practical methods for the synthesis of antileukemic drugs (Cladribine, Fludarabine, Nelarabine, Clofarabine) and a number of biologically important nucleosides were developed [7].

The enzymatic trans-ribosylation and trans-2-deoxyribosylation was used for the synthesis of the corresponding nucleosides of 5,6-difluorobenzimidazole and its derivatives, one fluorine atom of which is replaced with methoxy, ethoxy, isopropoxy, 4-morpholino and N-methylpiperazino groups aiming at search of new biologically active nucleosides. A large pocket close to the area corresponding to the purine N^1 atom was disclosed [18].

The MacDonald' method was studied for the synthesis of α-D-arabinofuranose-1-phosphate (Ara^F-α1P) using diverse starting peracyl derivatives of D-arabinose. Mixtures of different ratios of Ara^F-α1P and β-D-arabinopyranose-1-phosphate (Ara^P-β1P) were obtained and they will be used in the enzymatic condensations with purine and pyrimidine bases giving rise to the nucleosides, incl. Fludarabine, Nelarabine and 1-(β-D-arabinofuranosyl)-thymine. Similar approach was studied for the synthesis of antileukemic drug 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-2-chloroadenine (Clofarabine) [20,23].

An enzymatic synthesis of 2′-deoxyribonucleosides of 8-azapurines and 8-aza-7-deazapurines has been studied using the transglycosylation reaction and cascade one pot synthesis from 2-deoxy-D-ribose and nucleobases. Good substrate activity of 8-aza-7-deazapurinestowards recombinant E. coli purine nucleoside phosphorylase (PNP) was disclosed and mechanism of binding and activation of these analogs in the catalytic site of E. coli PNP was studied. The participation of Ser90·$O^γ$ of *E. coli* PNP in the binding of 8-aza-7-deazapurines in the catalytic center of PNP followed by the formation of productive complex and glycosidic bond was suggested [8] and proved by the preparation of the Ser90Ala mutant of *E. coli* PNP and investigation of its substrate properties [34].

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REFERENCES

1. *Mikhailopulo I. A.* Biotechnology of Nucleic Acid Constituents — State of the Art and Perspectives // Current Org. Chem. — 2007. — V. 11. — P. 317.
2. *Mikhailopulo I. A.,Miroshnikov A. I.* New Trends in Nucleoside Biotechnology // Acta Naturae. — 2010. — V. 2, N 5. — P. 36.
3. *Mikhailopulo I. A., Miroshnikov A. I.* Biologically important nucleosides: modern trends in biotechnology and application // Mendeleev Communs. — 2011. — V. 21. — P. 57.
4. *Doskocil J., Holy A.* // Coll. Czech. Chem. Communs. — 1977. — V. 42. — P. 370 (see also the works cited therein); *Votruba I., Holy A., Dvorakova H. et al.* // Ibid. — 1994. — V. 59. — P. 2303.
5. *Chuvikovsky D. V., Esipov R. S., Skoblov Y. S. et al.* // Bioorg. Med. Chem. — 2006. — V. 14. — P. 6327.
6. *Esipov R. S., Gurevich A. I., Chuvikovsky D. V. et al.* // Protein Expes. Purif. — 2002. — V. 24. — P. 56.
7. *Miroshnikov A. I., Esipov R. S., Konstantinova I. D. et al.* // The Open Conference Proc. J. — 2010.– V. 1. — P. 98.
8. *Stepchenko V. A., Seela F., Esipov R. S. et al.* Enzymatic Synthesis of 8-Azapurineand 8-Aza-7-deazapurine 2'-Deoxyribonucleosides // SynLett. — 2012. — V. 23. — P. 1541.
9. *De Clercq E.* // Adv. Virus Res. — 2009. — V. 73. — P. 1.

10. *Krosky P. M., Borysko K. Z., Nassiri M. R. et al.* // Antimicrob. Agents Chemother. — 2002. — V. 46. — P. 478; *Evers D. L., Komazin G., Shin D. et al.* // Antiviral Res. — 2002. — V. 56. — P. 61; *Gudmundsson K. S., Tidwell J., Lippa N. et al.* // J. Med. Chem. — 2000. — V. 43. — P. 2464.
11. *Prichard M. N.* // Rev. Med. Virol. — 2009. — V. 19. — P. 215; *Williams S. L., Hartline C. B., Kushner N. L. et al.* // Antimicrob. Agents Chemother. — 2003. — V. 47. — P. 2186; *Hwang J. S., Kregler O., Schilf R. et al.* // Virol. — 2007. — V. 81. — P. 11604; *Hwang J. S., Schilf R., Drach J. C. et al.* // Antimicrob. Agents Chemother. — 2009. — V. 53. — P. 5095.
12. *Trofe J., Pote L., Wade E. et al.* // Ann. Pharmacother. — 2008. — V. 42. — P. 1447; *Drugs R. D.* Maribavir: 1263W94, Benzimidavir, GW 1263, GW 1263W94, VP41263. — 2007. — V. 8 — P. 188; *Chou S., Marousek G. I.* // J. Virol. — 2008. — V. 82. — P. 246.
13. *Galmarini C. M., Popowycz F., Joseph B.* // Curr. Med. Chem. — 2008. — V. 15. — P. 1072; *Mathe C., Gosselin G.* // Antiviral Res. — 2006. — V. 71. — P. 276; *Biron K. K.* // Antiviral Res. — 2006. — V. 71. — P. 154.
14. *Mikhailopulo I. A., Zinchenko A. I., Bokut S. B. et al.* // Biotechnol. Letters. — 1992. — V. 14. — P. 885.
15. *Mikhailopulo I. A., Kazimierczuk Z., Zinchenko A. I. et al.* // Nucl. Acids Symp. Ser. — 1994. — N 31. — P. 83.
16. *Mikhailopulo I. A., Kazimierczuk Z., Zinchenko A. I. et al.* // Nucleosides Nucleotides. — 1995. — V. 14. — P. 477.
17. *Bentancor L., Trelles J. A., Nybile M. et al.* // J. Mol. Cat. B: Enzymatic. — 2004. — V. 29. — P. 3.
18. *Konstantinova I. D., Selezneva O. M., Fateev I. V. et al.* // Synthesis. — 2013. — V. 45. — P. 272.
19. (a) *MacDonald D. L.* // J. Org. Chem. — 1962. — V. 27. — P. 1107; (b) *MacDonald D. L.* // Carbohyd. Res. — 1966. — V. 3. — P. 117; (c) *MacDonald D. L.* // Carbohyd. Res. — 1968. — V. 6. — P. 376; (c) *Mendicino J., Hanna R.* // J. Biol. Chem. — 1970. — V. 245. — P. 6113; (d) *Chittenden G. J. F.* // Carbohyd. Res. — 1972. — V. 25. — P. 35.
20. *Konstantinova I. D., Antonov K. V., Fateev I. V. et al.* // Synthesis. — 2011. — V. 10. — P. 1555.
21. *Wright R. S., Khorana H. G.* // J. Am. Chem. Soc. — 1958. — V. 80. — P. 1994.
22. *Averett D. R., Koszalka G. W., Fyfe J. A.* // Antimicrob. Agents Chemother. — 1991. — V. 35. — P. 851.
23. *Fateev I. V., Antonov K. V., Konstantinova I. D. et al.* The Chemo-Enzymatic Synthesis of Clofarabine and Related Nucleosides. The Role of Electronic and Stereochemical Factors of Substrates in Reactions Catalyzed by *E. coli* Nucleoside Phosphorylases. Federation of European Biochemical Societies. CONGRESS 2013 «Mechanisms in Biology», July 6–11, 2013. — Sainct Petersburg, Russia. Abstracts.
24. *Roivainen J., Elizarova T., Lapinjoki S. et al.* // Nucleosides, Nucleotides & Nucl. — Acids. — 2007. — V. 26. — P. 905.
25. a) *Himmerlsbach F., Shulz B. S., Trichtinger T. et al.* // Tetrahedron. — 1984. — V. 40. — P. 59; b) *Zhou J., Tsai J.-Y., Bouhadir K., Slevin P. B.* // Synthetic Commun. — 1999. — V. 29. — P. 3003.
26. *Kulikovskiy A. I., Esipov R. S., Miroshnikov A. I., Mikhailopulo I. A.* An Enzymatic Synthesis of Nucleosides of N^2-Acetyl-O^6-[2-(4-nitrophenyl)ethyl]guanine // Collection Symp. Series. — 2011. — V. 12. — P. 397.
27. *Elion G. B.* // Cancer Res. — 1969. — V. 29. — P. 2448.
28. (a) *Seela F., Pujari S.* // Bioconjugate Chem. — 2010. — V. 21. — P. 1629; (b) *Jiang D., Seela F.* // J. Am. Chem. Soc. — 2010. — V. 132. — P. 4016.
29. *Seela F., Jiang D., Xu K.* // *Org. Biomol. Chem.* — 2009. — V. 7. — P. 3463, and references cited therein.
30. (a) *Pokharel S., Jayalath P., Maydanovych O. et al.* // J. Am. Chem. Soc. — 2009. — V. 131. — P. 11882; (b) *Veliz E. A., Easterwood L. M., Beal P. A.* // Ibid. — 2003. — V. 125. — P. 10867; (c) *Wierzchowski J., Ogiela M., Iwanska B., Shugar D.* // Anal. Chim. Acta. — 2002. — V. 472. — P. 63.
31. (a) *Elliot R. D., Montgomery J. A.* // J. Med. Chem. — 1976. — V. 19. — P. 1186; (b) *Kazimierczuk Z., Cottam H. B., Revankar G. R., Robins R. K.* // J. Am. Chem. Soc. — 1984. — V. 106. — P. 6379, and references therein.
32. (a) *Doskocil J., Holy A.* // Coll. Czech. Chem. Communs. — 1977. — V. 42. — P. 370 (see also the works cited therein); (b) *Wierzchowski J., Wielgus-Kutrowska B., Shugar D.* // Biochim. Biophys. Acta. — 1996. — V. 1290. — P. 9; (c) *Votruba I., Holy A., Dvorakova H. et al.* // Coll. Czech. Chem. Communs. — 1994. — V. 59. — P. 2303.
33. (a) *Koellner G., Luic M., Shugar D. et al.* // J. Mol. Biol. — 1998. — V. 280. — P. 153; (b) *Koellner G., Bzowska A., Wielgus-Kutrowska B. et al.* // J. Mol. Biol. — 2002. — V. 315. — P. 351; (c) *Bennett E. M., Li C., Allan P. W. et al.* // J. Biol. Chem. — 2003. — V. 278. — P. 47110; (d) *Zhang Y., Parker W. B., Sorscher E. J., Ealick S. E.* // Curr. Topics Med. Chem. — 2005. — V. 5. — P. 1259; (e)

Mikleusevic G., Stefanic Z., Narczyk M. et al. // Biochimie. — 2011. — V. 93. — P. 1610.
34. *Fateev I. V., Esipov R. S., Antonov K. V. et al.* The Role of the Ser90 Residue of the Catalytic Site of *E. coli* PNP in the Synthesis of Purine and 8-aza-7-deazapurine Nucleosides. Federation of European Biochemical Societies. CONGRESS 2013 «Mechanisms in Biology»; July 6–11, 2013. — Sainct Petersburg, Russia. Abstracts.

ДЕЯКІ НОВІ ДАНІ БІОТЕХНОЛОГІЇ БІОЛОГІЧНО АКТИВНИХ НУКЛЕОЗИДІВ

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Обговорено нещодавні відкриття в галузі біотехнології біологічно активних нуклеозидів, до яких належить низка лікарських препаратів проти лейкемії (кладрибін, флударабін, неларабін, клофарабін). Докладно розглянуто: нову стратегію каскадної «one-pot» трансформації D-пентоз у нуклеозиди, що ґрунтується на розширенні та поглибленні знань про механізм функціонування рибокінази, фосфопентомутази, уридин-, тимідин- і пуриннуклеозидфосфорилаз, а також роль різних чинників (структурних, електронних, стереохімічних) у формуванні глікозидного зв'язку; сучасні методи хемоензиматичного синтезу нуклеозидів; реакцію трансглікозилювання, що каталізується нуклеозидфосфорилазами, за якої донорами карбогідратних залишків виступають природні та модифіковані за цукрами нуклеозиди, а акцепторами — гетероциклічні основи.

Ключові слова: нуклеозиди, біоміметичний синтез, хемоензиматичний синтез, ензими метаболізму нуклеїнових кислот.

НЕКОТОРЫЕ НОВЫЕ ДАННЫЕ БИОТЕХНОЛОГИИ БИОЛОГИЧЕСКИ АКТИВНЫХ НУКЛЕОЗИДОВ

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Обсуждаются недавние открытия в области биотехнологии биологически активных нуклеозидов, к которым относится ряд лекарственных препаратов против лейкемии (кладрибин, флударабин, неларабин, клофарабин). Подробно рассмотрены: новая стратегия каскадной «one-pot» трансформации D-пентоз в нуклеозиды, основанная на расширении и углублении знаний о механизме функционирования рибокиназы, фосфопентомутазы, уридин-, тимидин- и пуриннуклеозидфосфорилаз, а также роль различных факторов (структурных, электронных, стереохимических) в формировании гликозидной связи; современные методы хемоэнзиматического синтеза нуклеозидов; катализируемая нуклеозидфосфорилазами реакция трансгликозилирования, при которой в качестве доноров карбогидратных остатков выступают природные и модифицированные по сахарам нуклеозиды, а акцепторов — гетероциклические основания.

Ключевые слова: нуклеозиды, биомиметический синтез, хемоэнзиматический синтез, энзимы метаболизма нуклеиновых кислот.