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Biologically important nucleosides: modern trends in biotechnology and application†

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Modern trends in biotechnology of nucleosides of biochemical and medicinal importance are outlined. Emphasis is made on the enzymatic transformations within the general chemo-enzymatic approach to the synthesis of nucleosides. Some aspects of (i) the functioning of enzymes of nucleic acid metabolism of interest as biocatalysts, (ii) the transglycosylation reaction using sugar modified nucleosides as donors of carbohydrate residues and heterocyclic bases as acceptors catalyzed by nucleoside phosphorylases and *N*-deoxyribosyltransferases, (iii) the miscellaneous transformations employing D-pentofuranose 5-phosphates or α-D-pentofuranose 1-phosphates as universal glycosylatig substrates, (iv) the retrosynthesis, and (v) the one-pot transformation of D-pentoses into nucleosides are considered.

Nucleosides embrace a large family of natural and chemically modified analogues of a great structural diversity and a broad spectrum of biological activity.[1–5] Analogues of natural nucleosides and 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 valuable constituents of artificial oligonucleotides of medicinal potential making these oligomers more stable in biological fluids and improving their targeting properties.

The vast majority of the modified nucleosides have been prepared by chemical methods. Despite the very impressive progress achieved in the development of chemical methods (*e.g.*, refs. 6,7), the preparation of many antiviral and anticancer drugs and biologically active compounds remains a challenging problem resulting in a high price of drugs and consequently preventing them from extensive biological trials and broad therapeutic application. The need for new strategies for the synthesis of nucleosides and their medicinal application became obvious in the late 1970s. Since then, progress was achieved in the development of new approaches to the synthesis of natural and modified nucleosides, the isolation of individual compounds and their use as antiviral and anticancer drugs or constituents of artificial oligonucleotides and substrates within the suicide gene therapy.

Here, we outline modern trends in the biotechnology of nucleosides and their medicinal applications. The biocatalyst technology typically replaces multistep chemical processes, and considerable

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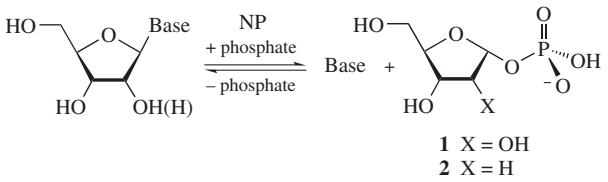
Professor **Anatoly I. Miroshnikov** (right) graduated from M. V. Lomonosov Institute of Fine Chemical Technology (Moscow) in 1963. In 1964–1983, he held various positions at the M. M. Shemyakin–Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences (Moscow); since 1991, he is deputy director of the institute. He received PhD and Dr. Sci. degrees in 1968 and 1989, respectively. In 1987–1991, he was the director of the All-Union Institute of Medicinal Plants (VILAR, Moscow). Professor Miroshnikov was elected a corresponding member and a full member of the Russian Academy of Sciences in 1994 and 2000, respectively. He is the chairperson of the Pushchino Scientific Centre (Moscow Region), a member of the Presidium of the Russian Academy of Sciences and Vice-President of the Russian Public Organization 'Society of Biotechnologists of Russia'. Professor Miroshnikov was awarded the Prizes of the Government of the Russian Federation in 1996 and 2005. His scientific interests cover synthetic organic and medicinal chemistry, the chemistry of natural compounds, the chemistry of oligonucleotides, the preparation of recombinant enzymes of nucleic acid metabolism and the biotechnology of nucleic acid components and hormones like insulin.

† This paper is dedicated to the 300th Anniversary of the birthday of M. V. Lomonosov, a great Russian researcher and historical personality.

progress in the preparation of nucleoside analogues was achieved by advisable combination of chemical methods and biochemical transformations. Emphasis is made on the enzymatic transformations within the general chemo-enzymatic approach to the synthesis of nucleosides. Some aspects of (i) the functioning of enzymes of nucleic acid metabolism of interest as biocatalysts, (ii) the transglycosylation reaction using sugar modified nucleosides as donors of carbohydrate residues and heterocyclic bases as acceptors catalyzed by nucleoside phosphorylases (NP) and *N*-deoxyribosyltransferases (DRT), (iii) the miscellaneous transformations employing D-pentofuranose 5-phosphates (PF-5P) or α-D-pentofuranose 1-phosphates (α-PF-1P) as universal glycosylating substrates, (iv) the retrosynthesis, and (v) the one-pot transformation of D-pentoses into nucleosides will be addressed.

Transglycosylation reaction

The transfer of a pentofuranosyl moiety of commercially available nucleosides or prepared by chemical methods to purine or pyrimidine bases catalyzed by NP or DRT (transglycosylation reaction) was shown to be an efficient methodology for the synthesis of a number of analogues of natural nucleosides of biological and pharmaceutical importance.[8–10] A donor of the pentofuranose residue may be either a natural nucleoside or its derivative and natural or artificial heterocyclic bases serve as acceptors.



Scheme 1 Reversible phosphorolysis of nucleosides catalyzed by nucleoside phosphorylases. 'Base' stands for the pyrimidine and purine bases.

The *E. coli* uridine (UP; EC 2.4.2.3), thymidine (TP; EC 2.4.2.4) and purine nucleoside (PNP; EC 2.4.2.1) phosphorylases are very important tools in the biotechnology of nucleosides.[8–10] Nucleoside phosphorylases catalyze *in vivo* the phosphorolysis of *ribo*- and 2′-*deoxyribo*-nucleosides in the presence of inorganic phosphate (P_i) giving rise to the formation of heterocyclic bases and 1-phosphates of α-D-ribofuranose **1** or 2-deoxy-α-D-ribofuranose **2**, and *vice versa* the condensation of 1-phosphates **1** and **2** with bases leading to the formation of nucleosides concomitant with P_i release (Scheme 1).[11] The equilibrium of the phosphorolysis reaction of pyrimidine nucleosides catalyzed by UP and TP is biased towards the product formation to a higher extent than in the case of purine nucleoside phosphorolysis catalyzed by PNP. 1-Phosphates **1** and **2**, which were produced by the catabolic action of NP, are involved in numerous biochemical transformations in living cells, *inter alia*, in the 'salvage' synthesis of therapeutically important pyrimidine [*e.g.*, 5-fluorouracil, (*E*)-5-(2-bromovinyl)uracil and purine (6-mercaptopurine, 6-thioguanine)] nucleosides.

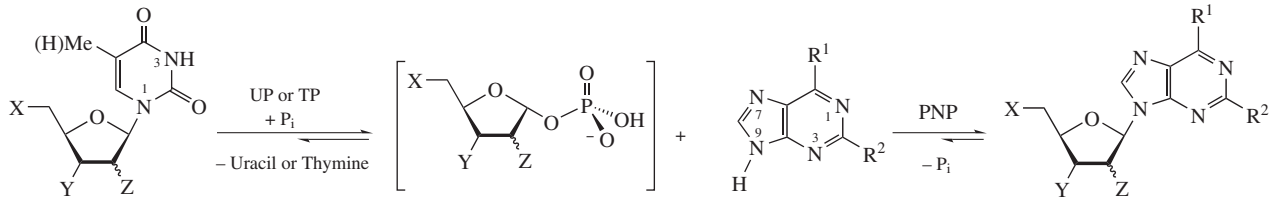
Pugmire and Ealick[12] performed a detailed structural analysis of NP and defined two distinct families of the enzymes, *viz.*, NP-I and NP-II.The first family includes homotrimeric and hexameric enzymes from both prokaryotes and eukaryotes that accept purine (inosine, guanosine and adenosine), as well as pyrimidine (uridine), nucleosides as substrates. The second family encompasses bacterial pyrimidine phosphorylases and eukaryotic thymidine phosphorylases. Note that mammalian TP was shown to be identical to the platelet-derived endothelial cell growth factor (PD-ECGF; also known as gliostatin[13,14]) stimulating endothelial cell proliferation[15] through the formation of 2-deoxy-D-ribose resulting from the thymidine phosphorolysis followed by the dephosphorylation of 1-phosphate **2**[15–17] (for a review, see ref. 18). This finding stimulated a search for TP inhibitors that could potentially be useful in the chemotherapy of cancers.[18–20]

There are two types of PNP, *viz.*, type 1 are hexameric enzymes (*e.g.*, *E. coli* PNP) and type 2 are trimeric enzymes (*e.g.*, human PNP).[12] In principle, both types of PNP can be used as biocatalysts for the synthesis of purine nucleosides. However, PNP from bacterial sources display a broader specificity *vs.* mammalian enzymes, accepting as substrates both 6-oxo- and 6-aminopurines and their nucleosides along with many analogues. TP reversibly catalyzes the phosphorolysis of thymidine and 2′-deoxyuridine, but not uridine and 1-(β-D-arabinofuranosyl)-thymine and -uracil, whereas UP does not distinguish between β-D-ribofuranose and 2′-deoxy-β-D-ribofuranose in pyrimidine nucleosides and also accepts 1-(β-D-arabinofuranosyl)pyrimidines as substrates; cytosine and its nucleosides are not substrates for both TP and UP[10] (*vide infra*). The results of pioneering studies clearly pointed to the possibility of an enzymatic synthesis of nucleosides from purine or pyrimidine heterocyclic bases employing either the α-PF-1P or another nucleoside as a pentofuranose donor (reviewed in refs. 9,10).

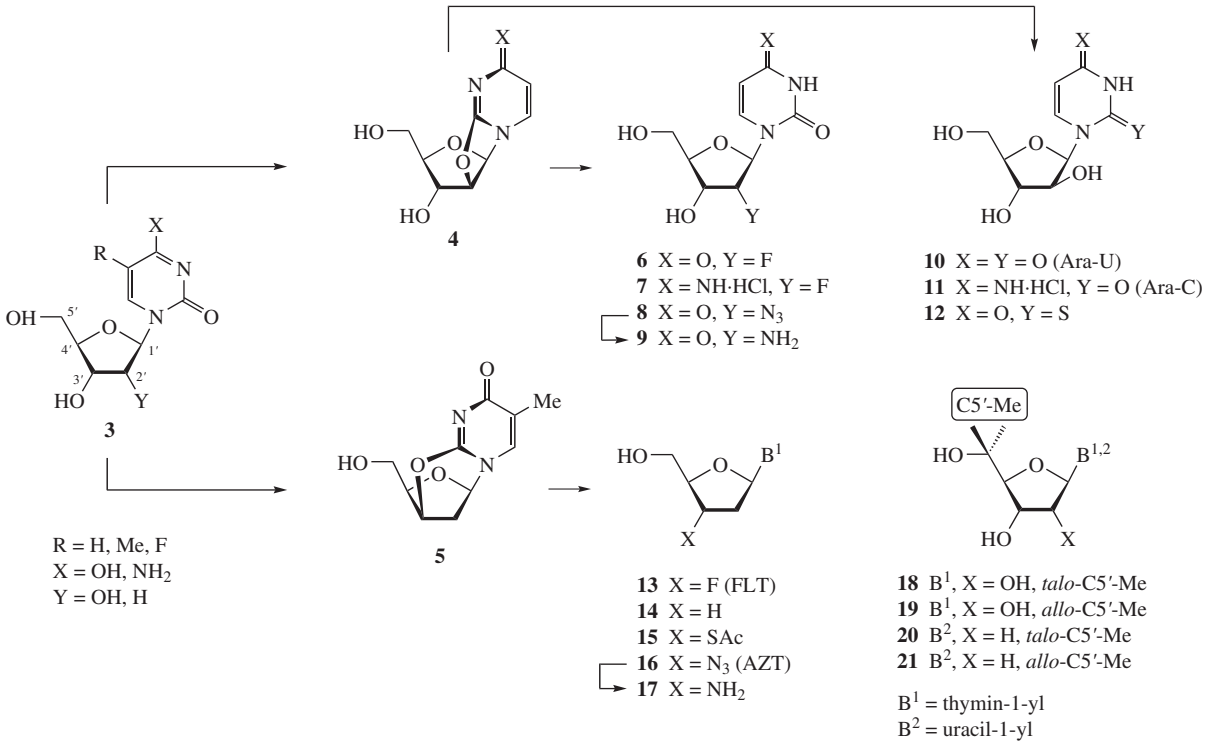
The most frequently exploited scheme of the transglycosylation reaction consists in the use of pyrimidine nucleosides as donors of pentofuranose residues and purine heterocyclic bases as acceptors (Scheme 2).

Note that the striking propensity of pyrimidine nucleosides **3** to the easy formation of O^2,2′- and O^2,3′-anhydro derivatives **4** and **5** by chemical methods, on the one hand, and their valuable, versatile reactivity, on the other, is a very important prerequisite for the use of sugar modified pyrimidine nucleosides as donors of the pentofuranose moiety in the transglycosylation reaction. Indeed, there are numerous very efficient chemical transformations of readily available natural *ribo* **3** (Y = OH, X = OH, NH_2, R = H, Me) and, to a lesser extent, 2′-*deoxyribo* **3** (Y = H, X = OH, R = Me) nucleosides in plethora analogues of biochemical, medicinal and biotechnological importance through an intermediary formation of these derivatives (Scheme 3).

A completely different situation exists in the case of natural purine nucleosides. First, they have no functions disposed to the



Scheme 2 Chemo-enzymatic synthesis of purine nucleosides using pyrimidine nucleosides as donors of a pentofuranose residue and nucleoside phosphorylases as biocatalysts in the transglycosylation reaction.



Scheme 3 Chemical synthesis of pyrimidine nucleosides that are of importance as donors (or as potential donors) in the transglycosylation reaction of purine heterocyclic bases catalyzed by nucleoside phosphorylases.

formation of similar *anhydro* derivatives. Second, the chemical introduction of keto or more useful thioketo function at C8 of purine nucleosides enables one to construct the 8,2′- or 8,3′-anhydro-8-oxy(mercapto) bridge altogether in four steps. Third, the anhydro derivatives thus obtained display remarkably lower reactivity *vs.* the corresponding pyrimidine O^2,2′- and O^2,3′-anhydro derivatives [*cf.*, the chemical syntheses of 9-(2,3-dideoxy-3-fluoro-β-D-ribofuranosyl) guanine (FLG) by the chemical transglycosylation of trimethylsilylated N^2-palmitoylguanine using 5′-*O*-acetyl-3′-deoxy-3′-fluorothymidine ($^{5'OAc}$FLT) as a donor of the sugar residue[21] and from guanosine through the intermediate formation of 8,2′-anhydro-8-mercaptoguanosine[22] *vs.* the chemo-enzymatic synthesis[23] of FLG (*vide infra*)]. Therefore, the use of a pentofuranose transfer path shown in Scheme 2 represents an advisable alternative to the chemical synthesis of sugar modified purine nucleosides from natural purine nucleosides.[9,10]

It is obvious that the substrate activity of sugar modified pyrimidine nucleosides for UP or/and TP is critically important factor in the development of efficient methods of the synthesis of desired purine and related nucleosides. On the other hand, an understanding of the mechanisms of substrate-enzyme binding and the contribution of different amino acids of the catalytic centre of enzyme to the activation of pyrimidine nucleosides serving as donors of the sugar fragments in the transglycosylation reaction are clues to both the selection of optimal donors and the creation of mutant enzymes with improved phosphorolytic activity. Recent publications have been devoted to the structure and mechanism of functioning of pyrimidine nucleosides and their substrate specificity. Thus, Caradoc-Davies and co-workers published the crystal structures of *E. coli* UP in two native and three complexed forms with 5-fluorouridine (^{5F}Urd; **3**, R = F; X = Y = OH), 2′-deoxyuridine (dUrd; **3**, R = H; X = OH, Y = H) and thymidine (Thd; **3**, R = Me, X = OH, Y = H) to gain insights into the mechanism of the enzyme-substrate interaction and its relationship to hexameric *E. coli* PNP.[24] In agreement with previous studies, it was found that *E. coli* UP shows a hexameric quaternary structure and significant structural homology with hexameric *E. coli* PNP.

The mode of binding ^{5F}Urd and phosphate (P_i) *vs.* those of dUrd/P_i and Thd /P_i are of interest from a viewpoint of the use of dUrd and Thd, as well as closely related nucleosides, as donors of pentofuranose residues in the transglycosylation reaction. In the former case, (i) the ribose C3′OH forms a hydrogen bond to one of the oxygen atoms of the phosphate ion; (ii) Glu198 forms a pair of hydrogen bonds to the C2′ and C3′ hydroxyl groups of the ribose moiety; (iii) the C2′ hydroxyl group forms hydrogen bonds to the main-chain nitrogen atom of Met197 and the side-chain of Arg91; (iv) His8 of the adjacent monomer forms hydrogen bond to the ribose C5′OH; (v) the four residues (His8, Arg91, Glu198 and Met197) are responsible for correct positioning of the ribose in the active site, they are identical in the human UP and conserved across all members of the NP-I family. The bindings of ^{5F}Urd/P_i, dUrd/P_i and Thd/P_i are similar and give rise to a strained conformation of nucleosides, *viz.*, the glycosidic bond conformation is forced to occupy the *anti* range of the base [+*anticlinal* (+*ac*) (*high-syn*) for ^{5F}Urd and dUrd; *antiperiplanal* (*ap*) for Thd; the torsion angles χ within *ca.* +110° to +160°] and rather unusual ^1E and $^1T_0$ (the phase angle of pseudorotation *P* within 280°–315°) accompanied by the close proximity of O5′ and O4′ oxygen atoms.[24]

Oliva *et al.*[25] characterized *E. coli* UP prepared by single-site mutagenesis. They found that (i) mutations Thr94Ala, Phe162Ala and Tyr195Gly give rise to a drastic decrease in UP activity, (ii) mutation of Glu198 to Gly, Asp or Gln causes complete loss of activity pointing to a critically important function of this amino acid both in the ribose binding and in interaction with P_i, and (iii) Arg91Ala and Glu196Ala lead to similar results. They pointed to the structural similarity between UP and PNP from *E. coli*. These data are very useful for the creation of mutant enzymes with improved phosphorolytic activity.

On going from ^{5F}Urd to dUrd and Thd, three hydrogen bonds to C2′OH are removed lowering the affinity of the latter to UP. The absence of the above hydrogen bonds can change the posi-

Table 1 Molecular structures of pyrimidine nucleosides from crystallographic studies.

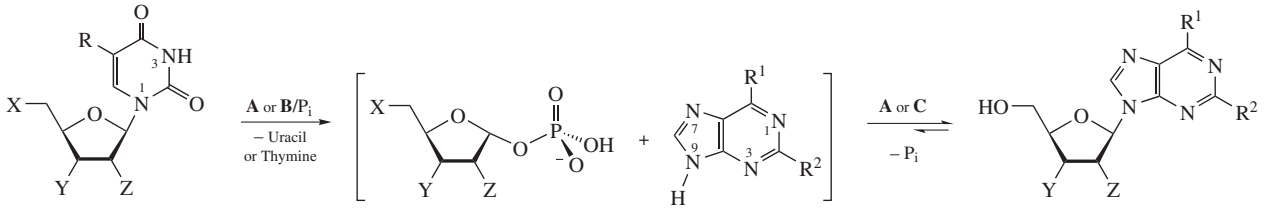
Compound	Bond lengths, Å			Base orientation[a] torsion angle χ/deg	Sugar ring conformation		Reference
	N1–C1′	C4′–O4′	O4′–C1′		(*P*; *N*,*S*)/deg	Ring puc-kering v_{max}/deg	
Urd **A**	1.497	1.453	1.414	–161.7	3.8 (*N*; $^3T_2$)	39.6	31
Urd **B**	1.483	1.454	1.412	–155.7	14.0 (*N*; ^3E)	41.7	
$Urd_{2'F}$	1.454 $\Delta^{Urd-Urd2'F}$ = =0.043±0.029	1.447	1.431	–133.4	70.8 (*N*; $^0T_4$, *East*)	38.2	32
dUrd **A**	1.450	1.430	1.420	–154.0	173.0 (*S*; $^2T_3$)	38.6	33
dUrd **B**	1.500	1.440	1.400	–155.0	177.9 (*S*; $^2T_3$)	36.5	
Thd	1.480	1.460	1.434	–140.0	C3′-*exo* (*S*)	—	34
FLT **A**	1.470	1.449	1.414	–133.4	164.3 (*S*; ^2E)	34.9	35
FLT **B**	1.474 $\Delta^{Thd-FLT}$ = = 0.010±0.005	1.446	1.409	–153.5	168.6 (*S*; $^2T_3$)	31.6	

[a]The χ angle is defined as the torsion angle O4′–C1′–N1–C2; an *anti* conformation of the bases around the glycosidic bond was found for all the nucleosides.

tions of the 2-deoxyribofuranose fragment and phosphate, hence explaining the decrease k_{cat} rate as it was recently manifested by a thorough comparative analysis of kinetic assays of recombinant *E. coli* UP[26] with pyrimidine nucleosides, *viz.*, the following K_M (10^{-6} M) and k_{cat} (s^{-1}) values were determined for Urd (80 and 98), dUrd (133 and 14) and Thd (270 and 5).[27] Note that a similar drop of substrate activity of dUrd *vs.* Urd had been observed for human UP.[28]

The replacement of the C2′OH group of Urd by a fluorine atom resulted in a dramatic loss of substrate activity of 2′-deoxy-2′-fluorouridine ($Urd_{2'F}$) for UP[29,30] and very low activity in regard to TP.[29] There is no obvious explanation of such strong differences in substrate activity if one takes into account that an electronegative fluorine atom could mimic the C2′ oxygen atom. However, a comparison of the crystallographic data for Urd and $Urd_{2'F}$ reveals the shortening of the glycosyl bond length of the latter pointing to strengthening of its glycosyl bond *vs.* Urd (Table 1). A similar trend is observed on going from Urd to dUrd and, moreover, sugar ring conformation is changed from *N* to *S*. Remarkably, the sugar ring of $Urd_{2'F}$ occupies in the crystal state rather unusual *N*-conformation close to the *E* (*East*) range. These structural peculiarities of dUrd and $Urd_{2'F}$ *vs.* Urd may contribute to the very low activity of the former along with the lack of the hydrogen bonds to C2′OH.

An interesting observation was made during the study on substrate specificity of UP and PNP of the whole cells of *E. coli* BM-11.[36] Thus, the relative activities of two pairs of regioisomeric analogues of Urd in the phosphorolysis catalyzed by UP were 35.5 and 6.4% for 2′-deoxyuridine (dU) **22** and 3′-deoxyuridine **23**, respectively, or 11.6 and 0.6% for 2′-amino-2′-deoxyuridine **9** and 3′-amino-3′-deoxyuridine **24**, respectively (an Urd activity was taken as 100%). In line with these data, the relative activities of the above uracil nucleosides as donors in the transglycosylation reaction of adenine were 78.8 and 4.9% for 2′- and 3′-deoxyadenosines (dA) **29** and **44**, respectively, or 2.7 and 0.2% for 2′-amino-2′-deoxy- and 3′-amino-3′-deoxyadenosines **30** and **31**, respectively. These data show that both *E. coli* enzymes, UP and PNP, reveal similar requirements to the structure of the C2′ and C3′ α-substituents of the pentofuranose ring modified substrates, and modifications at the C3′ impair the substrate activity to a greater extent in comparison with the same modifications at the C2′. The efficient syntheses of purine 2′-amino-2′-deoxy- and 3′-amino-3′-deoxy-nucleosides are described in a patent application[37] (Scheme 4).



	R	X	Y	Z	
9	H	OH	OH	NH_2	
17	Me	OH	NH_2	H	
22	H	OH	OH	H	(dU)
23	H	OH	H	OH	
24	H	OH	NH_2	OH	
25	H	H	OH	OH	
26	Me	OH	OH	H	(Thd)
27	Me	H	OH	H	
28	Me	NH_2	OH	H	

R^1	R^2	
NH_2	H	(Ade)
OH	NH_2	(Gua)
NH_2	NH_2	(DAP)
NHBz	H	
OH	NHAc	
NH_2	Cl	
OR	NHAc	

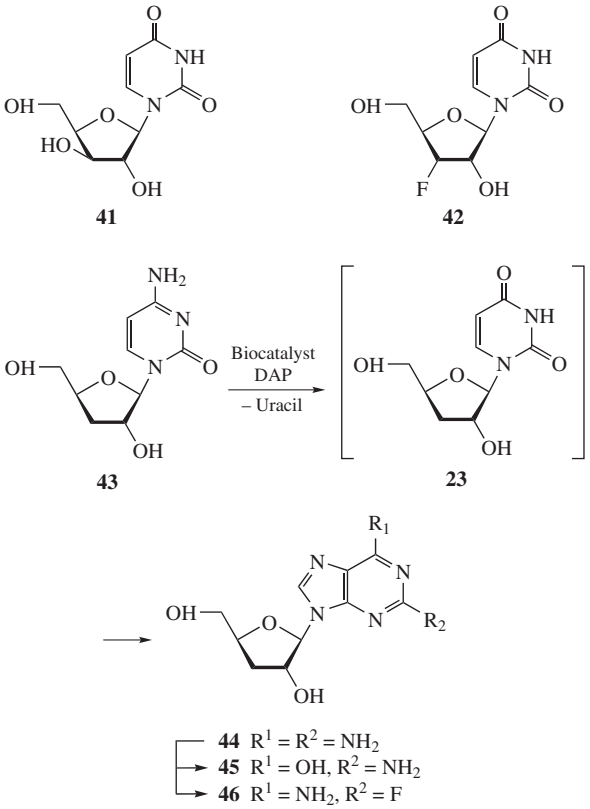
		R^1	R^2	Y	Z	
	29	NH_2	H	OH	H	(dA)
	30	NH_2	H	OH	NH_2	
	31	NH_2	H	NH_2	OH	
D	**32**	NH_2	NH_2	NH_2	OH	
	33	OH	NH_2	NH_2	OH	
D	**34**	NH_2	NH_2	OH	NH_2	
	35	OH	NH_2	OH	NH_2	
	36	NH_2	Cl	OH	H	
			(Cladribine)			
D	**37**	NH_2	NH_2	NH_2	H	
	38	OH	NH_2	NH_2	H	
	39	NHBz	H	NH_2	H	
	40	OR	NHAc	OH	H	
	(R = H, Me, benzyl)					

Scheme 4 Chemo-enzymatic synthesis of purine nucleosides employing pyrimidine nucleosides as donors of the pentofuranose residues, heterocyclic bases as acceptors and the whole bacterial cells or recombinant *E. coli* nucleoside phosphorylases as biocatalysts. **A** is the whole *E. coli* cells, **B** is the recombinant *E. coli* UP or TP, **C** is the recombinant *E. coli* PNP, **D** is adenosine deaminase.

The use of 2,6-diaminopurine (DAP) nucleosides, *e.g.* compounds **32**, **34** and **37**, as intermediates in the synthesis of the corresponding guanine nucleosides is advantageous over the use of very poorly soluble guanine as a substrate taking into account a satisfactory solubility of DAP, its excellent substrate activity for *E. coli* PNP[36] and quantitative deamination by adenosine deaminase (ADA) of DAP nucleosides into desired guanine derivatives, which usually precipitated from the reaction mixtures. Moreover, DAP nucleosides are valuable precursors for the synthesis of biologically important purine nucleosides such as 2-fluoroadenine and isoguanine.[9,10,39]

1-(β-D-Arabinofuranosyl)uracil (Ara-U) **10** revealed low activity for UP of the whole *E. coli* BM-11 cells (3.2%),[36] as well as for the recombinant *E. coli* enzyme[26] (K_M 5×10^{-4} M and k_{cat} 2.0 s^{-1}; *cf.* with relevant values for Urd, *vide supra*).[27] None the less, this level of activity was found satisfactory for the use of Ara-U as a donor of the β-D-arabinofuranose residue in the efficient syntheses of purine nucleosides.[9,10]

A number of pyrimidine nucleosides show extremely low substrate activity for *E. coli* UP thereby limiting the possibility of their application in the transglycosylation reaction. Among them are 1-(β-D-xylofuranosyl)uracil **41** and 3′-deoxy-3′-fluorouridine **42**,[36] 2′-C- and 3′-C-methyluridines.[36,38] The simplest explanation of the resistance of the glycosyl bonds of nucleosides **41** and **42** to the UP action might be the lack of the α-3′OH group, as well as the inability of the 2′-hydroxyl to compensate in part the functions of the 3′OH neighbour. However, it seems true only in part because 3′-deoxyuridine **23** (formed *in situ* from 3′-deoxycytidine **43**) is a good donor of the sugar residue in the transglycosylation of DAP using two types of the intact *E. coli* cells as biocatalysts (Scheme 5).[39] The selected *E. coli* BM-11 cells display high cytidine deaminase (CD) activity affording uracil nucleoside **23**, which served as a donor of the pentofuranose residue in the reaction catalyzed by UP and PNP of BM-11 and BMT-4D/1A *E. coli* cells to furnish 2,6-diamino-9-(3-deoxy-β-D-arabinofuranosyl)purine **44** in 64% isolated yield. The treatment of the latter with (i) ADA gave 3′-deoxyguanosine **45** (85%), and (ii) HF/HBF_4/$NaNO_2$ (Schiemam reaction) afforded 3′-deoxy-2-fluoroadenosine **46** (43%).



Scheme 5 Enzymatic synthesis of 2,6-diamino-9-(3-deoxy-β-D-ribofuranosyl)purine **44**, 3′-deoxyguanosine **45** and 3′-deoxy-2-fluoroadenosine **46**. Biocatalyst: the intact *E. coli* BMT-4D/1A & BM-11 cells (the latter cells display high cytidine deaminase activity; both types show high UP and PNP activities). **43**: DAP molar ratio 1.5:1.0, K-phosphate buffer (60 mM; pH 7.0), 52 °C, 26 h.

The lack of substrate activity of 2′-C- and 3′-C-methyluridines for *E. coli* UP is probably due to steric hindrances created by the β-methyl groups precluding the nucleosides from adopting the *high-syn* or *antiperiplanal* (*ap*) conformations in the substrate-enzyme complex, which seems necessary for the phosphorolytic cleavage of the glycosyl bond (*vide supra*). On the contrary, 1-(6-deoxy-β-L-talofuranosyl)uracil **18** and 1-(6-deoxy-β-D-allofuranosyl)uracil **19**,[36,38,40] as well as 5′-deoxyuridine **25**[27] and 5′-homouridine,[27] showed similar or even better substrate activity for *E. coli* UP in comparison to natural substrate, Urd, pointing to a large hydrophobic pocket at the exocyclic C5′OH group. Nucleosides **20** and **21** were used as donors of the sugar residues in the transglycosylation of adenine employing the whole *E. coli* BM-11 cells as a biocatalyst affording the respective adenine nucleosides in high yields.[38]

Recently, considerable attention was paid to structural studies of TP from both bacterial and, largely, mammalian sources. Some aspects of these studies are of interest from viewpoint of their application as biocatalysts for the synthesis of nucleosides.[41–44] TP is a dimer consisting of two identical subunits that composed of the small and large domains separated by a cleft. As distinct from *E. coli* UP, there are no crystal structures of *E. coli* TP in the complexed forms with natural substrate thymidine or its closely related analogues that hampers the precise information of the structure of the catalytic centre. However, an analysis of the native crystal structures of *E. coli* TP in three different crystallographic forms along with molecular modeling and *in vacuo* dynamics simulation methods allowed one to suggest a catalytic mechanism.[41,45] It is proposed that the binding of the substrates followed by domain movement gives rise to the closure of the active site cleft.[41,42,45]

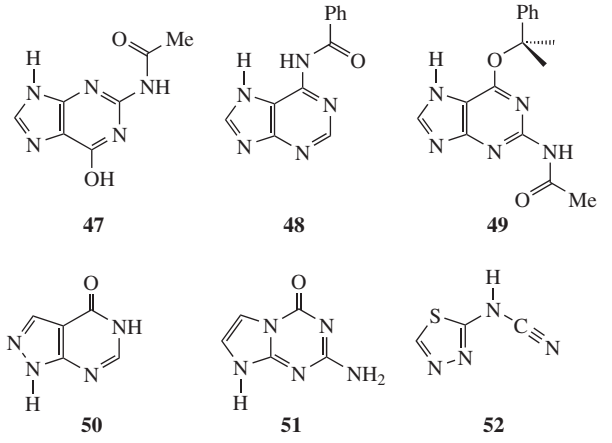
E. coli TP is a very important biocatalyst in the synthesis of purine and pyrimidine 2′-deoxy-β-D-ribosides.[9,10] The phosphorolytic cleavage of the sugar modified thymidine analogues is a key prerequisite for the realization of the *E. coli* TP potential as a biocatalyst for the synthesis of sugar modified nucleosides in the transglycosylation reaction. The substrate specificity of *E. coli* TP was recently studied in detail.[43,44] By analogy with *E. coli* UP, the C5′-hydroxyl group of thymidine is apparently not involved in binding and its replacement by a hydrogen atom does not lead to reduction of substrate activity of 5′-deoxythymidine; moreover, 5′-amino, 5′-mercapto and 5′-chloro functions are tolerated by the enzyme, whereas substitution of the iodide or azido group for the C5′-hydroxyl gives rise to the complete loss of substrate activity. 1-(2,6-Dideoxy-α-L-talofuranosyl)uracil **20** and 1-(2,6-dideoxy-β-D-allofuranosyl)uracil **21**, as well as 2′-deoxy derivatives of 5′-homouridine, showed satisfactory substrate properties for *E. coli* TP analogously to those found for corresponding parent nucleosides for *E. coli* UP[27] (*vide supra*).[44]

Replacement of the 3′-hydroxyl group by a hydrogen atom dramatically reduces the substrate activity of 3′-deoxythymidine **14** (K_M 32 mM, v_{max} 0.014 mM min^{-1}) *vs.* the parent thymidine (K_M 2.5 mM, v_{max} 5.2 mM min^{-1}).[43] It is somewhat puzzling that the amino function of 3′-amino-3′-deoxythymidine **17** fails to simulate the 3′-hydroxyl functions of thymidine resulting in a very low activity of **17** in the phosphorolysis reaction catalyzed by *E. coli* UP (Sigma-Aldrich).[43] Note that kinetic assays with recombinant *E. coli* UP[26] carried out by Mikhailov and co-workers[44] reveal a moderate inhibitory activity of nucleosides **14** and **17**. Similar discrepant results were obtained for uridine, which showed

very good substrate properties in assays with *E. coli* UP (56 and 81% of uridine was phosphrolysed in 1 and 24 h, respectively)[43] and displayed moderate inhibitory activity (K_I 0.06 mM *vs.* K_M 0.32 mM and k_{cat} 35 s^{-1} for 2′-deoxyuridine) in assays.[44] Despite the low substrate activity of 3′-amino-3′-deoxythymidine **17**, it was successfully used as a donor in the transglycosylation of purine bases.[37,46–48]

Overall, UP and TP complement one another allowing the design of the most efficient transglycosylation route towards the desired nucleoside. Note that enzymes that accept both thymidine and uridine are present in lower microorganisms, and they may be of interest as biocatalysts in the transglycosylation reaction. However, these enzymes have not yet found broad application in the synthesis of nucleosides.[10]

E. coli PNP is a key enzyme in the synthesis of purine and related nucleosides because it manifests a very broad substrate specificity.[8–10,49,50] The most important feature of *E. coli* PNP consists in its unique ability to accept not only diverse purine bases but also heterocyclic compounds in synthetic reaction (Scheme 6).[10] An examination of the structures of N^2-acetylguanine **47**, N^6-benzoyladenine **48** and acetamido-6-benzyloxypurine **49**, which displayed good substrate properties for *E. coli* PNP,[47] points to a large hydrophobic pocket at the N1–C2 fragment of the pyrimidine ring and even larger pocket at the C6 that can accommodate both the N^6-benzoyl and O^6-benzyl groups. Looking at the lower row of heterocyclic bases (Scheme 6), allopurinol **50**, 5-aza-7-deazaguanine **51** and *N*-(1,3,4-thiadiazol-2-yl)cyanamide **52**, which are also good substrates, one can suppose variable modes for the substrate-enzyme binding in the catalytic center of *E. coli* PNP. Taken together, these data illustrate a very impressive potential of *E. coli* PNP as a biocatalyst of the condensation of α-D-pentofuranose 1-phosphates with heterocyclic bases.



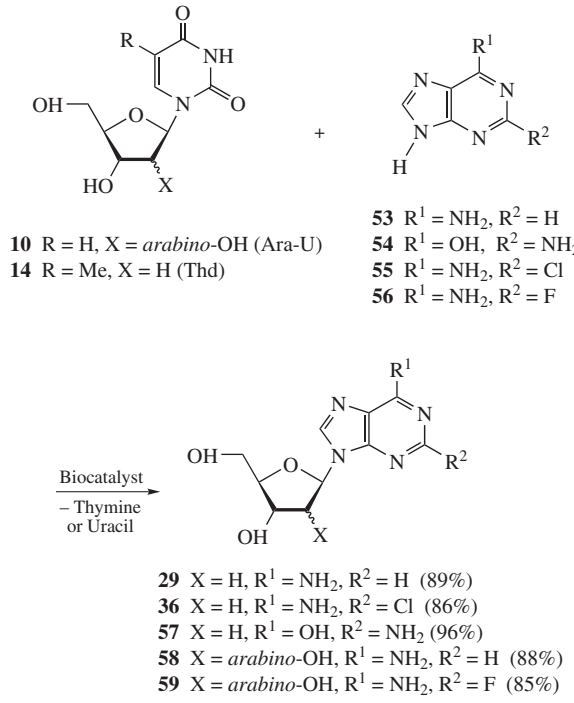
47 **48** **49**

50 **51** **52**

Scheme 6 Structures of some substrates of *E. coli* PNP.

Recently,[51] a new combination of partially purified *Bacillus halodurans* PNP and *E. coli* UP has been applied to the synthesis of 5-methyluridine from guanosine and thymine in a yield of 80% (*cf.* ref. 9). A paste of the *E. coli* cells overexpressed PNP, UP and TP have been used as a biocatalyst for the preparation of 2′-deoxyadenosine (dA) and 6-methyl-9-(2-deoxy-β-D-ribofuranosyl)purine in high yields.[52]

The use of nucleoside phosphorylases from thermophilic bacteria as biocatalysts in the transglycosylation reaction is advantageous over the *E. coli* and similar enzymes, *viz.*, enhancement of the reaction temperature from 50–55 (most frequently used with *E. coli* enzymes) to 70–75 °C results in an increase in (i) the concentrations of purine bases, which usually are poorly soluble substrates, and (ii) a rate of the transformation. Thus, natural and modified purine nucleosides have been synthesized using the recombinant thermostable purine nucleoside phosphorylase II (EC 2.4.2.1) and pyrimidine nucleoside phosphorylase (EC 2.4.2.2) from *Geobacillus stearothermophilus* B-2194 (Scheme 7).[53] The enzymes were produced in recombinant *E. coli* strains and covalently immobilized on aminopropylsilochrom AP-CPG-170 after heating the cell lysates and the removal of coagulated thermolabile proteins (see also ref. 54). The resulting preparations of thermostable nucleoside phosphorylases retained a high activity after 20 reuses in the transglycosylation reactions at 70–75 °C affording in high yields natural 2′-deoxy-β-D-ribonucleosides adenine (dA) **39** and guanine (dG) **57**, as well as pharmaceutically important 2-chloro-2′-deoxyadenosine (Cladribine) **36**, 9-(β-D-arabinofuranosyl) adenine (Ara-A, Vidarabine) **58** and 9-(β-D-arabinofuranosyl)-2-fluoroadenine (Fludarabine) **59**. The biocatalyst employed in this study showed high catalytic activity and thermal stability. It was simply filtered off from the reaction mixture, washed with a 50 mM K-phosphate buffer and repeatedly used in reactions.



10 R = H, X = *arabino*-OH (Ara-U)
14 R = Me, X = H (Thd)

53 $R^1 = NH_2$, $R^2 = H$
54 $R^1 = OH$, $R^2 = NH_2$
55 $R^1 = NH_2$, $R^2 = Cl$
56 $R^1 = NH_2$, $R^2 = F$

Biocatalyst
– Thymine
or Uracil

29 X = H, $R^1 = NH_2$, $R^2 = H$ (89%)
36 X = H, $R^1 = NH_2$, $R^2 = Cl$ (86%)
57 X = H, $R^1 = OH$, $R^2 = NH_2$ (96%)
58 X = *arabino*-OH, $R^1 = NH_2$, $R^2 = H$ (88%)
59 X = *arabino*-OH, $R^1 = NH_2$, $R^2 = F$ (85%)

Scheme 7 Enzymatic synthesis of purine nucleosides employing as biocatalyst recombinant PNP and PyNP from *Geobacillus stearothermophilus* enzymes immobilized on AP-CPG-170. Nucleoside–base molar ratio 3:1; 2–8 h, K-phosphate buffer (5 mM; pH 7.5), 75 °C [in the case of the dG (**58**) synthesis: K-phosphate buffer (5 mM; pH 10), 70 °C, 10 h]. HPLC yields are given.

Komatsu *et al.* (Mitsui Chemicals, Tokyo) disclosed a 2′-deoxycytidine (dC) producing enzyme (actually *E. coli* PNP)[23(a)] and Abe and co-authors[55] claimed the preparation of β-D-ribofuranosides, 2′-deoxy-β-D-ribofuranosides and 2′,3′-dideoxy-β-D-ribofuranosides of 5-azacytosine, cytosine and its 5-halogen and 5-alkyl derivatives. A suspension of the cell mass of phosphatase deficient *E. coli* strain producing PNP was mixed with 2-deoxy-β-D-ribofuranoside 1-phosphate (dRib-1P; dicyclohexylammonium salt) **2** in Tris·HCl buffer (100 mM, pH 8.0), frozen and stored at –20 °C. This preparation was used as a biocatalyst/dRib-1P component of the reaction mixture, and its activity was tested by the formation of 2′-deoxyadenosine (dA) after withstand of a portion at 30 °C for 1 h. Synthesis of 2′-deoxycytidine (dC) was conducted in 50% aqueous MeOH to inhibit the cytidine deaminase activity at a 1:1 molar ratio of dRib-1P/cytosine at 50 °C for 20 h leading to the desired nucleoside (53%, HPLC) along with 2′-deoxyuridine (*ca.* 1%) and uracil (*ca.* 0.5%). A few examples of the synthesis of cytosine nucleosides are given.[55]

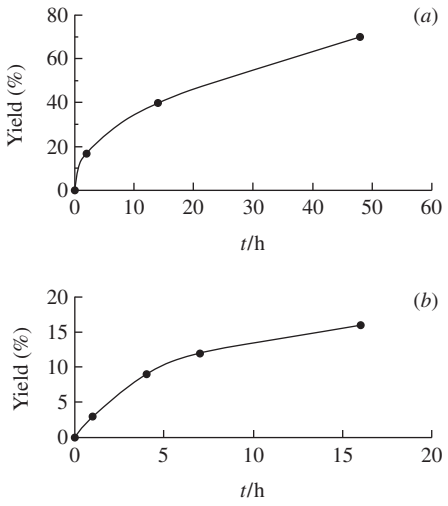


Figure 1 Phosphorolytic cleavage of (*a*) cytidine and (*b*) thymidine with PNP.

Notably, recombinant *E. coli* PNP that we used in our studies[26] was able to split the glycosyl bonds of cytidine and thymidine under very mild conditions (0.05 mmol nucleosides, 0.27 units[26] recombinant PNP; 80 mM K-phosphate buffer, pH 7.0; 14–15 °C) (Figure 1) and catalyze the synthesis of 2′-deoxycytidine and thymidine from 2′-deoxyguanosine.‡

Previously,[10] we paid attention to DRT as very important biocatalysts for the synthesis of base and sugar modified nucleosides. Here, we mention an exciting study by Kaminski and co-workers[56] on the preparation of the *Lactobacillus fermentum* NDT with an excellent activity for the transfer of β-D-dideoxypentofuranosyl residue from the corresponding nucleosides to pyrimidine and purine bases. They constructed random mutant libraries of *ndt* genes from *L. leichmanii* (Ll) and *L. fermentum* (Lf) with a variable frequency of nucleotide changes (between 1 and 10 per sequence), developed a functional screen method, and selected the variants specialized for the synthesis of 2′,3′-dideoxynucleosides. The nucleotide sequence of the corresponding genes revealed a single mutation (G → A transition), which caused a small aliphatic amino acid to be replaced by a residue with a hydroxyl group, Ala-15 by a Thr (Lf) or Gly-9 by a Ser (Ll), respectively. This single amino acid substitution was sufficient to enhance the substrate activity of 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydronucleosides for the mutant enzymes (Table 2).

These data illustrate the possibilities of improving and expanding the substrate specificity of *N*-deoxyribosyltransferases aiming at the development of practical methods for the preparation of pharmaceutically important nucleosides.

Table 2 Substrate activity (*U*/mg of protein) of NDT *Lactobacillus fermentum* wild type (WT) and A15T with thymine (T), adenine (A) and cytosine (C) acceptors and 2′,3′-dideoxy-β-D-ribonucleosides of cytosine, adenine and thymine as well as 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) as donors.

Enzymatic transformation	*Lactobacillus fermentum* NDT		
	WT	A15T mutant	A15T/WT ratio
ddC + T → ddT	<0.01	1.47	>147
ddC + A → ddA	0.06	7.10	118
ddA + T → ddT	<0.01	0.42	>42
ddA + C → ddC	0.03	1.35	45
ddT + A → ddA	0.03	0.50	16.6
d4T + A → d4A	0.036	9.28	250

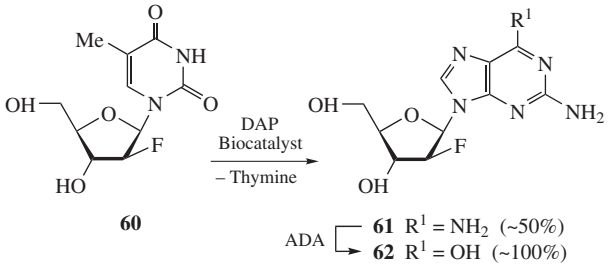
‡ V. A. Stepchenko, F. Seela, R. S. Esipov, A. I. Miroshnikov and I. A. Mikhailopulo, in preparation.

Miscellaneous transformations (employing α-D-pentofuranose 1-phosphates as universal glycosylatig substrates)

Krenitsky *et al.*[57,58] had patented the preparation of purine nucleosides employing 3′-deoxy-3′-fluorothymidine (FLT) **13** and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (FMAU) **60** as donors of the corresponding sugar residues and *E. coli* TP absorbed on DEAE cellulose catalyzing phosphorolytic cleavage of the glycosyl bonds. A common feature of these syntheses is their low efficiency owing to the laborious preparation of FLT and FMAU and a very low substrate activity for *E. coli* TP. Consequently, very large amounts of the enzymes and long reaction times are required to perform the enzymatic transfer of the sugar moiety and to achieve acceptable yields. Thus, the synthesis of 2,6-diamino-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)purine **61** from arabinoside **60** (1.2 mmol) was carried out in the presence of very large amounts of *E. coli* TP (160 000 IE) and PNP (290 000 IE) (Scheme 8).[58] Note that the deamination of DAP arabinoside **61** by ADA afforded 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine **62** in a quantitative yield.

The lack of the C3′ hydroxyl group in FLT molecule appears to be the main case of its very low substrate activity for *E. coli* TP. Indeed, the glycosidic bond lengths of thymidine and FLT, as well as the stereochemistry of pentofuranose rings, are similar (Table 1). On the other hand, note that the pentofuranose ring of FLT is rigid and populated in the C3′-*exo* conformation in solution[59] that can be hardly compatible with the spatial arrangement of the substrate in the catalytic center of *E. coli* TP.[41] The very low substrate activity of arabinoside **60** for *E. coli* UP is likely due to steric hindrances created by the C2′-β-fluorine atom preventing the nucleoside from the formation of a productive substrate-enzyme complex.

Deoxyfluoro pyrimidine and purine nucleosides under consideration attracted much attention since the first synthesis of FLT,[60] FMAU[61] and 9-(2,3-dideoxy-3-fluoro-β-D-ribofuranosyl) guanine (FLG) **65**[21(a)] because they show interesting biological activity[4,5,62,63] and arabinosides **60**–**62** are very valuable constituents of oligonucleotides (for a review, see ref. 64). The use of universal glycosylating agents that are suitable for glycosylation of both pyrimidine and purine bases is the most productive strategy to develop practical methods for the preparation of these nucleosides. In the case of 2,3-dideoxy-3-fluoro-β-D-ribofuranosyl nucleosides, a number of sugar derivatives suitable for the glycosylation of heterocyclic bases have been described.[65] However, the use of these glycosylating agents for the chemical synthesis of nucleosides usually leads to the formation of mixtures of α- and β-anomers and, moreover, of the regioisomers in the case of purine bases resulting in the preparation of the individual desired compounds in low combined yields after tedious chromatographic separations. Similar situation is in the case of 2-deoxy-2-fluoro-β-D-arabinofuranosyl nucleosides, where commercially available



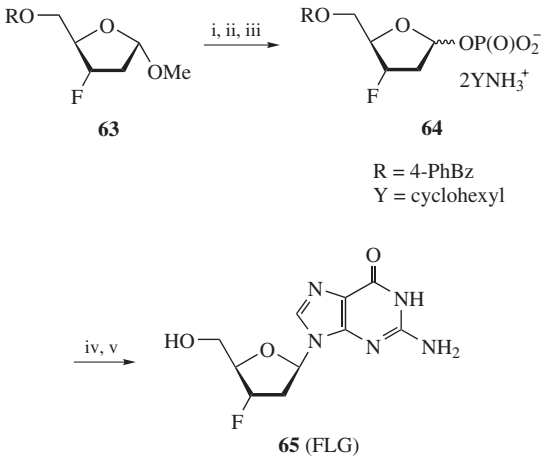
60

Scheme 8 Enzymatic synthesis of purine nucleosides from FMAU **60**. Biocatalyst: *E. coli* TP (160 000 IE per 1.2 mmol **62**) and PNP (290 000 IE) absorbed on DEAE cellulose, K-phosphate buffer (5 mM; pH 7.0), 37 °C, 48 h; 50 °C, 120 h; AG 1×2 (OH^--form) ion exchange chromatograpy followed by silica gel column chromatography (×2). DAP is the 2,6-diaminopurine (excess), ADA is the adenosine deaminase.

1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranose **66** found broad application in the synthesis of nucleosides,[64,65] including Clofarabine, a drug against blood malignances.[66]

The α-D-pentofuranose 1-phosphates (PF-1Ps) are reasonable alternative universal glycosylating agents to the chemical synthesis of pyrimidine and purine nucleosides. During recent years, the use of PF-1Ps as substrates of an enzymatic synthesis of nucleosides attracted much attention.[9,10] Note that the enzymatic and chemical syntheses of D-ribofuranose 1-phosphate and its 2-deoxy counterpart have a reach prehistory.[9,10] However, only recently a few reports concerning potential practical applications have been published. There are two lines of investigation in this area, *viz.*, (i) the chemical synthesis of PF-1Ps followed by the enzymatic condensation with heterocyclic bases,[67–70] and (ii) the biochemical (microbial, enzymatic) retrosynthesis of 2′-deoxyribonucleosides (*vide infra*).

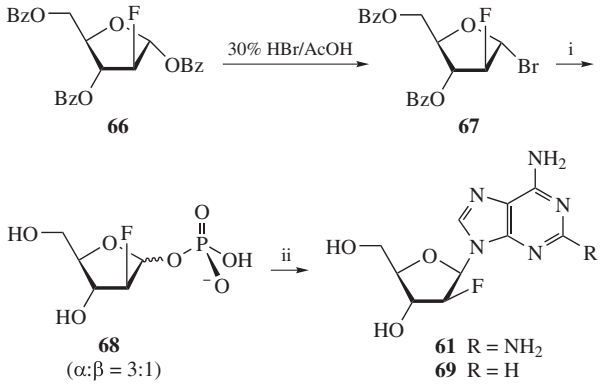
The chemo-enzymatic synthesis consisting of the chemical synthesis of PF-1Ps (usually as a mixture α,β-anomers) followed by enzymatic condensation with heterocyclic bases seems attractive for the preparation of sugar and base modified nucleosides of biological and medicinal values. However, most of the methods for the PF-1P preparation are laborious and low yielding. Moreover, in the case of deoxyfluoro sugars, glycosylating agents of the chemical synthesis of nucleosides or their precursors are starting compounds for the preparation of PF-1Ps. Thus, in the synthesis of FLG, methyl glycoside **63** was transformed in three steps into a mixture of α,β-anomers (87:13) of 1-*O*-phosphate **64** followed by deprotection and condensation with guanine afforded the desired product in good yield (Scheme 9).[68,69(a)] It is obvious that methyl glycoside **63** is a valuable glycosylating substrate for an enzymatic synthesis of diverse nucleosides.



Scheme 9 Chemo-enzymatic synthesis of FLG **65** employing *E. coli* PNP. *Reagents and conditions*: i, Ac_2O/AcOH/H_2SO_4, 0 °C, 2.5 h; ii, 4 N HCl/1,4-dioxane, 0 °C, 8 h; iii, *o*-H_3PO_4/Bu_3N, 4A MS, MeCN/4-methylpentan-2-one (10:1), 0 °C, 6 h, then *c*-$C_6H_{11}NH_2$ (Σ 59%); iv, aq. KOH, 50 °C, 48 h; v, guanine/PNP (sugar/guanine molar ratio 1:0.84), $CaCl_2$, 40–60 °C, 5 h (Σ 63%).

A similar approach was described by Yamada *et al.*[70] for the stereoselective synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)purines. Benzoate **66** was transformed into bromide **67**, which reacted with a phosphorylating mixture and then debenzoylated to give a 3:1 mixture of the α- and β-anomers of 2-deoxy-2-fluoro-α-D-arabinofuranose 1-phosphate **68**. This mixture was employed in the condensation with purine bases catalyzed by PNP from *Bacillus stearothermophillus*, and desired nucleosides **69** and **63** were obtained in 29 and 39% yield, respectively (Scheme 10).

The preparation of phosphate **68** was studied in detail and optimal reaction conditions, which can be employed for the synthesis of related phosphates, were proposed.[70] Moreover, essentially lesser amount of *B. stearothermophillus* PNP compared to the transglycosylation reaction[58] (Scheme 8) was necessary to achieve satisfactory yields of purine nucleosides **69** and **61**; the latter was deaminated by ADA to corresponding guanine nucleoside **62** in 87% yield.

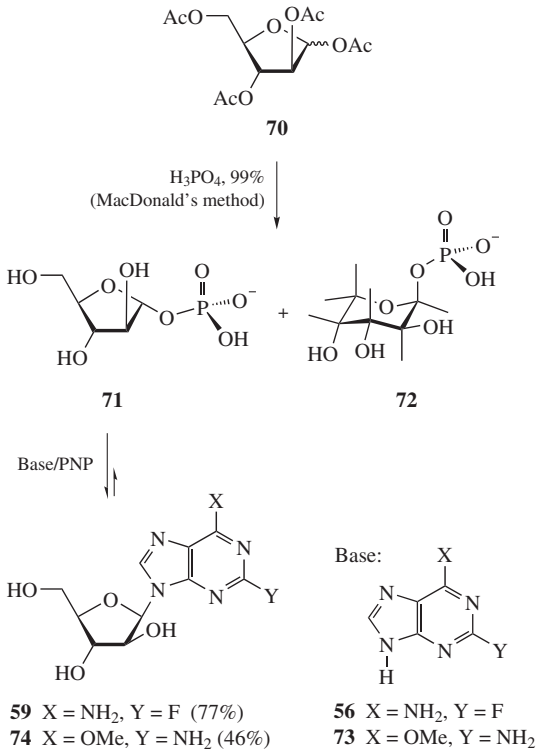


Scheme 10 Chemo-enzymatic synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)purines **61** and **69** employing PNP *B. stearothermophollus*. *Reagents and conditions*: i, diverse reaction conditions; ii, adenine (4 mmol)/PNP (2000 units), 20 mM K-phosphate buffer (pH 7.6), 50 °C, 144 h, membrane filtration, ODS column chromatography (Σ 29% from **67**); 2,6-diaminopurine (27 mmol)/PNP (23460 IU), 5 mM K-phosphate buffer (pH 7.6), 50 °C, 190 h (Σ 39% from **67**).

Scrutiny of the chemical methods for the preparation of pento-(hexo)furanose 1-phosphates as well as different methods of the activation of an anomeric carbon atom (for a review, see ref. 71) shows that the MacDonald method seems to be the most effective.[72] This method was mainly used for the synthesis of pyranose 1-phosphates. Therefore, it is of interest to study its feasibility in the preparation of pentofuranose 1-phosphates. It was found that treatment of 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose **70** with anhydrous phosphoric acid under conditions described by MacDonald[72] followed by work-up resulted in the formation of a viscous or powdered mixture consisting of α-D-arabinofuranose 1-phosphate (AraF-α1P) **71** and β-D-arabinopyranose 1-phosphate (AraP-β1P) **72** (*ca.* 50%, combined; the **71**:**72** ratio was 1.5:8 to 1:2). The formation of pyranose phosphate **72** along with expected furanose isomer **71** may be explained by the higher thermodynamic stability of the former. As might be expected, pyranose phosphate **72** showed neither substrate nor inhibitory activity for *E. coli* PNP, and the mixture of both isomers was used in the reaction with purine and pyrimidine bases. Condensation of AraF-α1P [as a 1:2 mixture with AraP-β1P (3 mmol)] with 2-fluoroadenine **56** (0.3 mmol) in aqueous solution in the presence of *E. coli* PNP (1.0 IU) was conducted at 55 °C for 1 h. During this time, 5–6% of the base remained in the reaction mixture, it was cooled, set into a thermostat at 14 °C and stored for 24 h; pure 9-(β-D-arabinofuranosyl)-2-fluoroadenine (Fludarabine) **59** was deposited from the reaction mixture (Scheme 11).[73]

An enzymatic condensation of 2-amino-6-methoxypurine **73** with AraF-α1P under similar reaction conditions proceeded very slowly and reached equilibrium after 36 h at almost equimolar concentrations of the base and its arabinoside (Nelarabine) **74**, which was isolated in 40% yield.[73]

In summary, note that the lack of a reliable method for the synthesis of pentofuranose 1-*O*-phosphates is a serious bottleneck of this technique. However, despite the rather complicated synthesis of PF-α1Ps this chemo-enzymatic approach to biologically valuable nucleosides represents an advisable alternative to an enzymatic transfer of a sugar fragment of nucleoside to heterocyclic base (transglycosylation reaction).

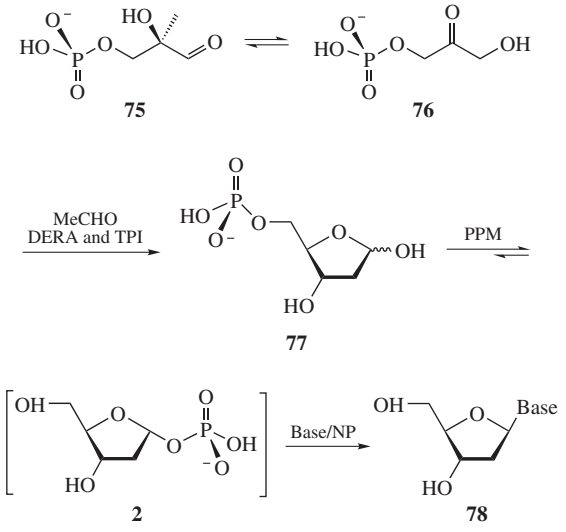


Scheme 11 Chemo-enzymatic synthesis of 9-(β-D-arabinofuranosyl)purines **59** and **74** from AraF-α1P **71** employing recombinant *E. coli* PNP.

Retrosynthesis of nucleosides

During last decade, studies were devoted to the retrosynthesis of 2′-deoxyribonucleosides.[74–76] The approach includes three consecutive enzymatic transformations: (i) condensation of D-glyceraldehyde-3-phosphate (Gla-3P) **75** or dihydroxyacetone monophosphate (DHAP) **76** and acetaldehyde catalyzed by D-2-deoxyribose-5-*O*-phosphate aldolase (DERA) and triose phosphate isomerase (TRI) affording D-ribofuranose 5-*O*-phosphate **77**, (ii) stereospecific transformation of the latter into α-D-ribofuranose 1-*O*-phosphate (RF-α1P) **2**, and (iii) condensation of RF-α1P with heterocyclic bases catalyzed by nucleoside phosphorylases (Scheme 12).

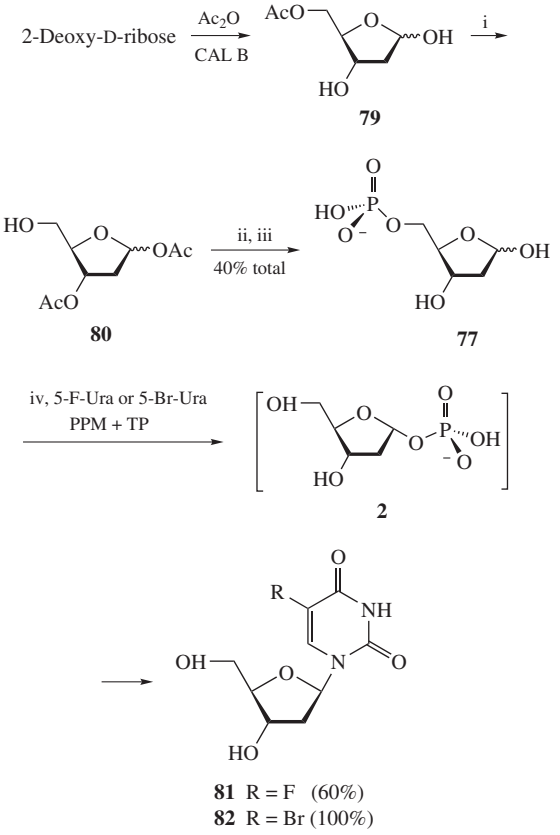
A number of modifications of this general retro-pathway including (i) chemical synthesis of DHAP[74] and the alcoholic fermentation system of baker's yeast as a source of Gla-3P,[75]



Scheme 12 General retro-pathway for nucleoside synthesis starting from Gla-3P **75** or DHAP **76** and acetaldehyde.

(ii) selection of the *Klebsiella pneumoniae* B-4-4 strain tolerated high concentrations of acetaldehyde, which directs the reversible DERA-catalyzed reaction towards the 5-phosphate **77** synthesis, (iii) selection of transformed *E. coli* pTS17/BL21 cells, expressing *E. coli* phosphopentomutase (PPM), which stereospecifically catalyzes the translocation of the 5-phosphate of **77** into 1-phosphate **2**, and selection of the DERA expressing *E. coli* 10B5/pTS8, which transforms D-fructofuranose 1,6-diphosphate (FDP) into an equilibrated mixture of phosphates **75** and **76**.[75,76] It is obvious that this approach to the synthesis of 2′-deoxyribonucleosides is very interesting. However, some problems should be solved to develop a universal practical method for the synthesis of natural 2′-deoxyribonucleosides that are main precursors in the synthesis of oligonucleotides. Thus, the presence of adenosine deaminase (ADA) in the whole cells used as biocatalysts leads to deamination of 2′-deoxyadenosine formed.

Recently, a very interesting variant of the retrosynthesis of nucleosides was described by Montserrat *et al.*[77] They developed an efficient chemo-enzymatic synthesis of the 5-*O*-phosphates of D-ribose, 2-deoxy-D-ribose and D-arabinose. The *Candida antartica* B lipase (CAL B) was used as a biocatalyst of selective 5-*O*-acetylation of 2-deoxy-D-ribose to lock it in the furanose form. Monoacetate **79** thus prepared was acetylated and then selectively 5-*O*-deacetylated using CAL B to afford diacetate **80**. The latter was transformed into 5-*O*-phosphate **77**, which was used as a universal glycosylating substrate in the tandem enzymatic synthesis of pyrimidine nucleosides (*e.g.*, fluoride **81** and bromide **82**) employing PPM and TP as biocatalysts (Scheme 13); a similar approach was used for the synthesis of β-D-ribofuranosyl and -arabinofuranosyl nucleosides.[77]



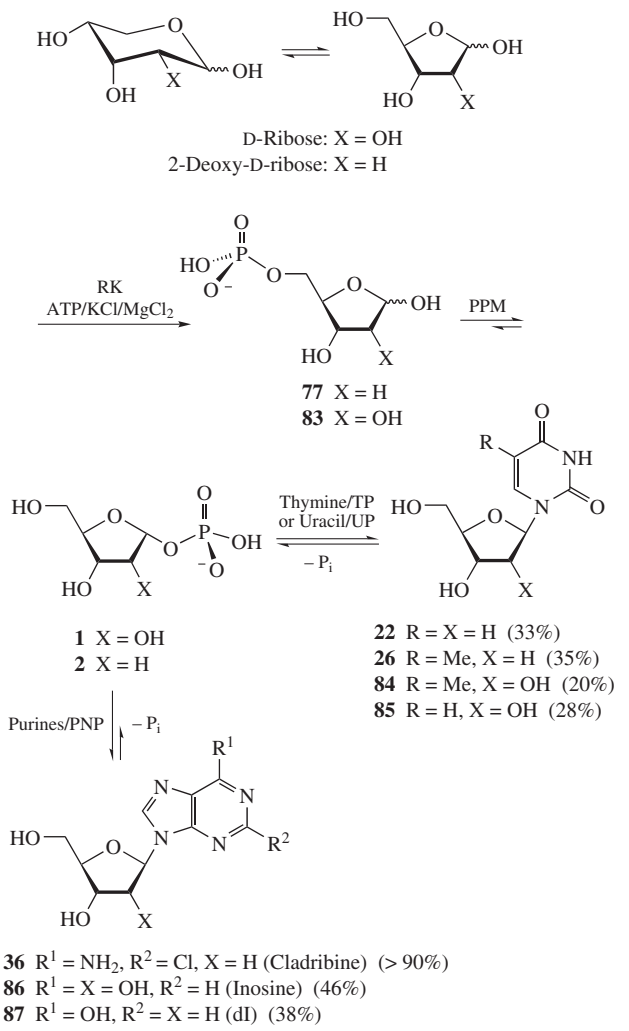
Scheme 13 The retrosynthesis of pyrimidine nucleosides starting from chemically prepared 2-deoxy-D-pentofuranose 5-*O*-phosphate **77**. *Reagents and conditions*: i, Ac_2O/Py, then EtOH, CAL B; ii, $(BzO)_2P–NPr^i_2$/tetrazole/THF, then Bu^tOOH; iii, H_2 (gas)/$Pd(OH)_2$/C, then ion-exchange chromatography ($Ba^{2+} \rightarrow Na^+$ change); iv, **81** (1 mM)/PPM + glucose-1,6-diphosphate (0.5 mM)/TP (Sigma–Aldrich enzyme), $HOCH_2CH_2SH$ (50–100 mM), Tris-buffer (pH 7.0).

Note that the use of the whole cells expressing DERA and PPM as biocatalysts in the synthesis of 5-*O*-phosphate **77** and catalyzing its transformation into 1-*O*-phosphate **2** appears limited to the production of 2′-deoxy-β-D-ribonucleosides.[75,76] In contrast, the approach suggested by Montserrat *et al.*[77] essentially expands the possibility of the retrosynthesis because it is not limited to the preparation of 2′-deoxyribonucleosides.

One-pot transformation of D-pentoses into nucleosides

Recently, we suggested a new strategy for the synthesis of nucleosides.[78] It consists in the cascade transformation of D-pentoses into nucleosides employing recombinant *E. coli* ribokinase[78] (RK) [D-pentoses → D-pentofuranose-5-phosphates (D-PF-5P)], phosphopentomutase[79–81] PPM [D-PF-5P → α-D-pentofuranose-1-phosphates (α-D-PF-1P)], and nucleoside phosphorylases[26] (NPs) (α-D-PF-1P + heterobase → nucleosides) in the presence of pyrimidine or purine heterobases. We found that, under optimum conditions, RK catalyzes the phosphorylation of the primary hydroxyl group of not only D-ribose and 2-deoxy-D-ribose but also D-arabinose and D-xylose. These data prompted us to consider this reaction as the first stage in a cascade transformation of pentoses into nucleosides (Scheme 14).[79–81]

Analysis of the optimal reaction conditions for RK,[78] PPM[80,82] and recombinant nucleoside phosphorylases[26] revealed essential differences. Bearing this in mind, we optimized the one-pot reaction conditions aiming at the finding out a compromised composition of the components allowing satisfactory function of the test enzymes. Further experiments were performed in one-pot fashion using D-ribose or 2-deoxy-D-ribose as starting pentoses, thymine, uracil, hypoxanthine and 2-chloroadenine as bases, and the RK, PPM and relevant NP as biocatalysts. Note that an equimolar ratio of D-pentose and heterocyclic base employed under the standard reaction conditions is not optimal. Optimization of the ATP/2-chloroadenine/2-deoxy-D-ribose ratio in the synthesis of 2′-deoxy-2-chloroadenosine led to the desired product in a yield of >90% (Scheme 14).



Scheme 14 One-pot transformation of D-pentoses into nucleosides. *Standard reaction conditions*: equimolar (2 mM) ratio of D-pentose and base, 2 mM ATP, 50 mM KCl, 3 mM $MnCl_2$, 20 mM Tris·HCl (pH 7.5), 20 °C; enzymes (respective units): RK, 7.65; PPM, 3.9; TP, 4.5; UP, 5.4; PNP, 4.68. HPLC yields are given. The ratio of ATP/2-cloroadenine/2-deoxy-D-ribose 0.9:0.5:0.75.

As we have not observed an enhancement of PPM activity in the synthesis of uridine **85** from D-ribofuranose 5-phosphate **83** and uracil in the presence of D-glucose 1,6-diphosphate [*cf.* ref. 82(*a*)] all further experiments have been performed without any 1,6-diphosphates. The formation of inosine **86** under the standard reaction conditions proceeded slightly faster *vs.* that of 2′-deoxyinosine **87** reaching maximum yields after 30 min (46%) and 24 h (38%), respectively. In contrast to this, we have earlier found that the *trans*-2′-deoxyribosylation of purines and deazapurines proceeds with higher efficiency than the *trans*-ribosylation (*e.g.*, ref. 9). The formation of thymidine **26** proceeded with higher efficacy (yield, 35%) compared to that of 1-(β-D-ribofuranosyl) thymine **84** (20%). However, the final concentrations of uridine **85** (28%) and 2′-deoxyuridine **22** (33%) were rather similar. On the whole, the concerted action of RK, PPM and PNP in one-pot transformation needs a careful optimization of the reaction conditions to achieve an optimal yield of the desired nucleoside.

In conclusion, all the above results demonstrate the progress in enzymatic methods for nucleoside synthesis. An analysis of the chemo-enzymatic syntheses of nucleosides clearly indicates that this methodology is highly effective and very promising for the development of biotechnological processes. The biocatalyst technology typically replaces multistep chemical processes, and considerable progress in the preparation of nucleoside analogues was achieved by advisable combination of chemical methods and biochemical transformations.

A number of very interesting findings was made recently in this field. In particular, Christoffersen *et al.*[83] described the one-pot synthesis of 2′,3′-dideoxyinosine (*ca.* 30% yield) from 2′,3′-dideoxyuridine and hypoxanthine using *Clostridium* phosphorylases as biocatalyst. A miraculous phosphopentomutase from *Thermococcus kodakaraensis* (TK2104) displayed a maximum activity at 90 °C (pH 7.5) with a specific activity of 210±10 μmol min^{-1} mg^{-1}.[84,85] It is obvious that this PPM is of interest as a biocatalyst in diverse enzymatic syntheses.

Nucleoside phosphorylases are of importance as not only biocatalysts for the synthesis of nucleosides but also targets for the treatment of diverse disorders. Thus, a detailed investigation into the mechanism of PNP functioning[86] resulted in the design of Forodesine (Immucillin H), which is the most potent PNP inhibitor and is undergoing the clinical trials for the treatment of hematologic malignancies.[87] The gene-directed enzyme prodrug therapy (suicide gene therapy) is another example of the use of nucleoside phosphorylases and modified nucleosides. This therapeutic strategy is based on the transformation of nontoxic prodrugs into powerful cytotoxic drugs in tumor cells that are genetically modified to express suicide genes and produce enzymes capable of releasing a drug. A combination of *E. coli* PNP gene and purine nucleosides is one of the most promising systems in suicide gene/prodrug therapy.[88–90] Nucleosides of 6-methylpurine and 2-fluoroadenine are not substrates of human PNP, whereas *E. coli* PNP can split these nucleosides releasing 6-methylpurine or 2-fluoroadenine that are very toxic for mammalian cells. The *E. coli* PNP gene transfer into tumor cells treated with the relevant

nucleosides followed by the selective expression of the enzyme results in the release of 6-methylpurine or 2-fluoroadenine and the inhibition of tumor cells.

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