Enzymatic Synthesis of 2′-Deoxy-β-D-ribonucleosides of 8-Azapurines and 8-Aza-7-deazapurines

Vladimir A. Stepenenko,* Frank Seela, Roman S. Esipov, Anatoly I. Miroshnikov, Yuri A. Sokolov, Igor A. Mikhailopulo* 

Abstract: The enzymatic synthesis of 8-azapurine and 8-aza-7-deazapurine 2′-deoxyribonucleosides has been studied. Two methods have been used: (i) transglycosylation employing 2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyuridine, and 2′-deoxythymidine as 2′-deoxy-D-ribofuranose donors and recombinant E. coli purine nucleoside phosphorylase (PNP) as biocatalyst, and (ii) one-pot synthesis from 2′-deoxy-D-ribose and nucleobases employing recombinant E. coli ribokinase (RK), phosphopentomutase (PPM) and PNP as biocatalysts. Good substrate activity was observed for all bases studied except 2-amino-8-aza-6-chloro-7-deazapurine, which afforded the desired N′-nucleoside in moderate yield due to very low solubility of the base and partial replacement of C6-chloro atom of the base and formed nucleoside with a hydroxy group. The participation of Ser90 O of E. coli PNP in the binding of 8-aza-7-deazapurines in the catalytic center of PNP followed by the formation of a productive complex and glycosidic bond is suggested.

Keywords: azapurine, nucleosides, enzyme catalysis, glycosylation, phosphorylases

Nucleosides with 8-azapurines and 8-aza-7-deazapurines (purine numbering throughout) are applicable as drugs and tools in chemistry, chemical biology, and molecular diagnostics. Chemical syntheses of these nucleoside shape mimics suffer from the formation of mixtures of isomers, which makes it necessary to conduct time-consuming separation procedures. As a result, the desired nucleosides are obtained in moderate or low yields (for example see refs. and those in the Supporting Information).

Nucleoside phosphorylases (NP), in particular E. coli NP, are very efficient biocatalysts in glycosylation reactions. Doskocil and Holy have shown that 8-azaguanine is a good ribosyl acceptor in the enzymatic reaction catalyzed by E. coli purine nucleoside phosphorylase (PNP; product of deoD gene; EC 2.4.2.1). Later, Votruba et al. studied alginate 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 purine and pyrimidine bases including aza and deaza analogues. The reaction proceeds regio- and stereospecifically, affording 8-aza-2′-deoxyadenosine and 8-aza-2′-deoxyguanosine. Furthermore, it was found that the presence of nitrogen-7 of purines and their isosteric analogues is a prerequisite for the reaction. However, there are several exceptions; 5-aza-7-deazaguanine (1), 5-aza-7-deazaisoguanine (2) and N-(1,3,4-thiadiazol-2-yl)-cyanamide (3) are substrates for bacterial purine nucleoside phosphorylases (Figure 1).

We applied the enzyme-catalyzed glycosylation to diverse range of heterocyclic bases belonging to the classes of 8-azapurines and 8-aza-7-deazapurines. The work presented here reports on the use of various donors of the 2′-deoxy-D-ribofuranosyl residue and recombinant E. coli PNP as a biocatalyst (transglycosylation reaction; Scheme 1, path A) and enzymatic cascade transformation of 2′-deoxy-D-ribose into the nucleosides (Scheme 1, path B). This cascade reaction uses recombinant E. coli ribokinase (RK), phosphopentomutase (PPM) and PNP (one-pot synthesis). We studied the enzymatic synthesis of N′-2′-deoxy-β-D-ribonucleosides of a number of 8-azapurines (4 and 5) and 8-aza-7-deazapurines (6–9) (Figure 2).

First, the substrate specificity of bases 4–8 was tested in the transglycosylation reaction performed under standard conditions using 2′-deoxyguanosine (10) as a glycosyl donor (Scheme 1, path A). All the bases are satisfactory and the respective nucleosides were obtained in good yields. The structure of these nucleosides (15–19) was confirmed by 1H, 13C NMR and UV spectroscopic analysis and compared to previously published data (see the Supporting Information). We did not observe the formation of regioisomeric nucleosides.

Figure 1 Some unusual substrates of E. coli purine nucleoside phosphorylase in synthetic reactions

SYNLETT 2012, 23, 1541–1545
Advanced online publication: 29.05.2012
DOI: 10.1055/s-0031-1290679; Art ID: ST-2012-B0090-L
© Georg Thieme Verlag Stuttgart·New York
The regiospecific 2'-deoxy-D-ribofuranosylation of 8-azaadenine (4) and 8-azaguanine (5) catalyzed by recombinant E. coli PNP is in line with published data. In contrast, glycosylation of anions of 8-azapurines with 2-deoxy-3,5-di-O-(4-toluyl)-α-D-erythropentofuranosyl chloride gave a very complex mixture of regioisomers and their α,β-anomers. There are a great number of publications devoted to the tautomerism of 8-azapurines and the chemical reactivity of nitrogen atoms of nucleobase anions that are involved in the formation of the glycosyl bond (reviewed in refs. 20, 21). The nucleobase anions are one of the activated species in the chemical glycosylation of 8-azapurins existing in a mixture of different tautomers; the other method of activation of purine bases involves the preparation of trimethylsilyl derivatives, -C–N\(^{+}\), leading to the enhancement of nucleophilicity of nitrogen atoms of the sp\(^2\) hybridized imino -C=N\(^{\#}\). An interplay of the equilibrium of tautomeric forms of anions or imino tautomers, on the one hand, and nucleophilicity of the activated nitrogen atoms, on the other, are responsible for the formation of regioisomeric nucleosides. In the case of the E. coli PNP catalyzed coupling of base and pentofuranosyl, activation of natural purines involves fixing a specific sp\(^2\) hybridized imino -C=N\(^{\#}\) tautomer of base in the catalytic site. From a chemical viewpoint, the formation of the glycosyl bond results from nucleophilic attack of a nitrogen atom of the base on the electrophilic C1-carbon atom of 2-deoxy-D-ribofuranose 1-phosphate (14). The regioselectivity of the enzymatic glycosylation is governed by the binding mode of base in the catalytic center of the enzyme enabling nucleophilic attack of a nitrogen atom of the sp\(^2\) hybridized imino -C=N\(^{\#}\) tautomer on the electrophilic center of the co-substrate. An analogous mode of binding can be assumed with high probability for natural purine bases as well as for 8-azapurines 4 and 5, leading to the exclusive formation of N\(^{\#}\)-glycosides. However, the formation of nucleosides 17–19 was rather unexpected in the light of the earlier data pointing to the crucial importance of nitrogen-7 of purines and their isosteric analogues in the synthetic reaction catalyzed by E. coli PNP.

The mechanism of the synthetic reaction catalyzed by E. coli PNP nucleoside phosphorylases has not attracted the attention of researchers thus far, and many important details are not clear (see, for example the discussion in the literature). Thus, the mode of binding of the substrate or inhibitor in the catalytic center of the enzyme might shed light on the mechanism of the enzyme’s functioning, and provide clues that could help to explain some unusual observations such as the good substrate activity of 8-aza-7-deazapurines. 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. It is noteworthy that replacement of D-aspartic acid residue 204 by alanine resulted in complete loss of the catalytic activity of E. coli PNP (see note on page 1266 of the paper by Ealick et al. and the recent paper by Luic et al.). Taking into account that it is an equilibrium reaction, one can expect that the same amino acid residues make the main contribution to binding of the substrate in the synthetic reaction. Asp204 interacts with nitrogen-7 and the substituent at C6 of the purine base, giving rise, in all likelihood, to the optimal base orientation and to enhancement of the nucleophilic properties of nitrogen-9, i.e., activation of the substrate (Figure 3). It is, therefore, surprising that replacement of nitrogen-7 with a CH group did not abolish the substrate activity of bases 6–8, which suggests a rather efficient contribution of the Asp204–C6 substituent interaction to correct binding and activation. To establish the role of such interaction, we investigated the substrate properties of base 9, which has no groups that could imitate an interaction of natural purine substrates with Asp204. It was surprising to find that base 9 still retains

**Scheme 1** Synthesis of nucleosides 15–20 by the transglycosylation reaction (path A) and cascade one-pot transformation of 2-deoxy-D-ribose into nucleosides (path B)
moderate substrate activity, despite the absence of any interactions with Asp204 and the extremely low solubility.

![Figure 3](https://example.com/figure3.png)

**Figure 3** Schematic presentation of binding adenine, hypoxanthine and guanine (22), followed by activation (23), and a possible binding of 2-amino-8-aza-6-methoxy-7-deazapurine (7) and 5-aza-7-deazaguanine (1); the most populated tautomers are show for bases in structures 24 and 25.

It appears clear that binding of 2-amino-8-aza-6-methoxy-7-deazapurine (7) in the catalytic center of PNP cannot lead to activation of the substrate. Indeed, analysis of the tautomeric structures of base 7 by ab initio calculations using a Polack–Ribiére conjugate gradient (HyperChem 8.01) showed a strong preference for the sp²-hybridized amino -C=N(H)- tautomer.23,24 Remarkably, similar analyses using a semi-empirical method PM3 (in water box) for three possible tautomeric structures of 5-aza-7-deazaguanine (1) revealed a strong preference for the sp³-hybridized imino -C=NH- tautomer (Figure 3).7b This electronic structure is in harmony with satisfactory substrate activity of isoseric guanine analogue 1, implying the important role of Asp204 and Phe159 residues in correct binding at the catalytic center of *E. coli* PNP.

Analysis of the crystal structure of the ternary complex of hexameric *E. coli* PNP with formycins A and B showed that Ser90 is involved in binding of the bases.23,24 Moreover, crystallographic data for adenine binding to the active site of *E. coli* PNP clearly showed the close proximity of Ser90 Oγ to carbon-8 of the base.25,26a These data, together with the aforementioned considerations, suggest a possible explanation for the good substrate properties of bases 6-8 and moderate activity of base 9; it is apparent that Ser90 Oγ is hydrogen bonded to nitrogen-8 (purine numbering) of the base in 26, giving rise to the correct base orientation in the catalytic site of *E. coli* PNP, followed by activation of the nitrogen-9 in productive complex 27 (Figure 4). The contribution of Phe159 of *E. coli* PNP to 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 analogues, the Ser90 residue of the catalytic site of *E. coli* PNP takes the place of Asp204 in the case of natural purine substrates.

The spatial tautomeric structures of base 9 in complex with Ser90-Oγ have been analyzed by the restricted Hartree–Fock (RHF) ab initio method using basis set of 6-31** FIREFLY QC package,29 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 optimization30 was used as starting approximation for the ab initio calculations. The following main dimensions were obtained for the respective structures 26: Ser90-Oγ···Nδ (0.97 and 1.86 Å); Ser90-Oγ···Nδ (2.39 and 0.99 Å); =Nδ···Nσ (1.39 Å); 27: Ser90-Oγ···Nδ (2.37 and 0.99 Å); Ser90-Oγ···Nδ (0.96 and 1.83 Å); =Nδ···Nσ (1.37 Å); these data are in fair agreement with strong hydrogen bonding of the base, enabling correct binding followed by activation.

In the next series of experiments, we studied the one-pot synthesis of nucleosides 15–19 with 2-deoxy-d-ribose and heterocyclic bases 4–8 in the presence of recombinant RK, PPM and PNP (Scheme 1, Path B).7b,16 Under the reaction conditions employed, the formation of 8-aza-2′-deoxyadenosine (15) and 8-aza-2′-deoxyguanosine (16) proceeded slowly, affording the nucleosides in moderate yields. In contrast, 8-aza-7-deazapurines 6–8 showed satisfactory substrate activity and the respective nucleosides 17–19 were formed in yields of more than 50% after 20 hours (Scheme 1; see also the Supporting Information).

Recently, Mitsui Chemicals, Inc. reported an enzymatic method for the production of nucleosides.32 Surprisingly they found that recombinant *E. coli* PNP is able to catalyze the reaction of cytosine and 2-deoxy-d-ribofuranose 1-phosphate in Tris-HCl buffer (100 mM, pH 8.0; 50 °C, 20 h) giving rise to the formation of 2′-deoxyctydine (11; dC) in 57.5% yield (HPLC). We have confirmed this finding and found that dC is phosphorylated under very mild conditions (nucleoside 0.05 mmol; 80 mM K,Na-phosphate buffer, pH 7.0; 27 units14 PNP; 14–15°C), resulting in more than 60% product yield (Figure 5). Furthermore, 2′-deoxyuridine (12; dU) and, to a lesser extent, 2′-deoxythymidine (13; dT) are also cleaved. However, a plateau is reached in the phosphorylation of dT implying two competing reactions: phosphorylosis and glycosylation. It is noteworthy that Pugmire and Ealick performed detailed structural analysis of NP and defined two distinct families of the enzymes: NP-I and NP-II.33 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. The activity of *E. coli* PNP against dU, dC and dT deserves detailed structural analysis. It should be stressed that the corresponding ribonucleosides showed no substrate activity with *E. coli* PNP under the aforementioned reaction conditions.

In conclusion, efficient enzymatic methods for the preparation of *N*′-*2*-deoxy-β-D-ribonucleosides of 8-azapurines and 8-aza-7-deazapurines are described. One of the approaches involves pentofuranose transfer from *2*-deoxyguanosine or *2*-deoxyxycytidine to heterocyclic bases catalyzed by recombinant *E. coli* PNP. The use of the one-pot transformation of *2*-deoxy-β-D-ribose into the aforementioned nucleosides through intermediate formation of *2*-deoxy-β-D-ribofuranose 5-phosphate (21), *2*-deoxy-α-D-ribofuranose 1-phosphate (14), and finally nucleosides catalyzed by recombinant *E. coli* RK, PPM and PNP, respectively. The methods employed in the present work for the synthesis of *2*-deoxy-β-D-ribonucleosides are complementary to each other. We tested both methods to assess, first of all, the feasibility of the latter for the synthesis of base-modified nucleosides. It is clear that the choice of method depends on the availability of the enzymes and the starting pentose or its donor.

The participation of Ser90 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 the glycosidic bond is suggested. The reaction conditions in both types of enzymatic reactions have not been optimized. Despite this, the heterocyclic bases 4–8 showed satisfactory substrate properties and the desired *N*′-*2*-deoxy-β-D-ribofuranosyl nucleosides have been prepared in good yields.

**Acknowledgment**

Financial support by the International Science and Technology Centre (project #B-1640) is gratefully acknowledged. I.A.M. is deeply thankful to the Alexander von Humboldt-Stiftung (Bonn–Bad-Godesberg, Germany) for partial financial support of this study. The authors thank Drs I.D. Konstantinova, K.V. Antonov, and I.V. Fateev (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences) for repeating some experiments and for performing analyses on the reaction mixtures.

**Supporting Information** for this article is available online at http://www.thieme-connect.com/ejournals/loc/synlett.

**References and Notes**

2'-Deoxy-β-d-ribonucleosides of 8-Azapurines and 8-Aza-7-deazapurines

(15) (a) Bennett, E. M.; Li, C.; Allan, P. W.; Parker, W. B.; Ealick, S. E. J. Biol. Chem. 2003, 278, 47110.
(17) Among the 8-aza-7-deazapurines studied in the present
work, the chemical and the physical properties of allopurinol (8) (the neutral molecule as well as cation and anion) have been an object of numerous studies (see for example refs. 20 and 28). Notably: (1) the neutral molecule is present as a mixture of tautomeric forms with predominant population of the keto =N=N(H)-tautomer [46 (purine numbering)]; (2) the anion of allopurinol is also present as a mixture of tautomeric forms and yields upon methylation N8,N9-, N8,N9,N10-, and N8,N9,N10-dimethyl derivatives [26].
(23) Pugmire, M. J.; Ealick, S. E. Biochem. J. 2002, 361, 1. The following reaction conditions employed in these experiments: the ratio of donor to acceptor was 1:1.0 (mol), 10 mM K Na-phosphate buffer (pH 7.0) containing 10% (v/v) DMSO; 1000 units PNP per 1.0 mmol base, reaction temperature was 50 °C. The progress of the nucleoside formation was followed by HPLC column HYPER SIL 150 × 4.6 mm, 5 μm; eluent: H2O; 1 mL/min; tR = 4.1 (4), 6.0 (15), 2.0 (6), 5.9 (17) min.