

PLENARY PRESENTATIONS

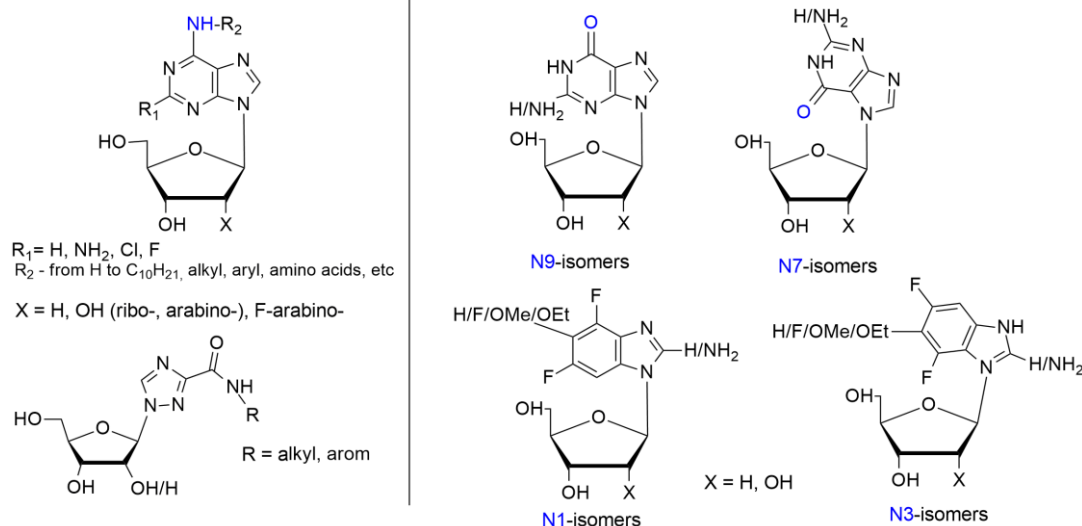
WHY ARE THE NON-SPECIFIC REACTIONS CATALYZED BY *Escherichia coli* PURINE NUCLEOSIDE PHOSPHORYLASE NOT EXPLAINED BY CLASSICAL CATALYSIS MECHANISMS?

Irina D. Konstantinova*, Ilya V. Fateev, Maria Ya. Berzina, Barbara Z. Eletskeya, Alexey L. Kayushin, Roman S. Esipov, Anatoly I. Miroshnikov

Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya St. 16/10, 117997, Moscow B-437, Russian Federation.

**e-mail: kid1968@yandex.ru*

E. coli purine nucleoside phosphorylases (PNP) are often used in the enzymatic synthesis of modified nucleosides. It is known that the transglycosylation reaction is characterized by high stereo- and regio-selectivity and is environmentally friendly. The active sites of *E. coli* and humans PNPs have been studied for several decades. A mechanism for the catalytic process has been proposed based on X-ray structural analysis data. However, we know numerous examples of a regioselectivity lack in the synthesis of purine, fleximer, and modified benzimidazole nucleosides.



We have found that the synthesis of N9-modified nucleosides proceeds regioselectively only with purines having amino groups in the C6 positions of purine. Thermodynamically unfavorable N7 isomers of natural inosine and guanosine appear in the transglycosylation reaction. In the case of substituted benzimidazole bases, a mixture of N1-, N3-regioisomers is always formed in the transglycosylation reaction. In the case of synthesis of 1,2,4-triazole nucleosides, the glycosylation regioisomerism by N1 atom is preserved in 99% of cases. We observed the largest number of errors in the synthesis of nucleosides in the case of glycosylation of fleximer heterocyclic bases in which the purine and pyrrole/pyrazole rings are separated by a single C-C bond. It was in the case of enzymatic synthesis of fleximer nucleosides that we found nucleosides with two carbohydrate residues.

We will try to clarify the mechanism of catalysis to explain all cases of the regioselectivity lack.