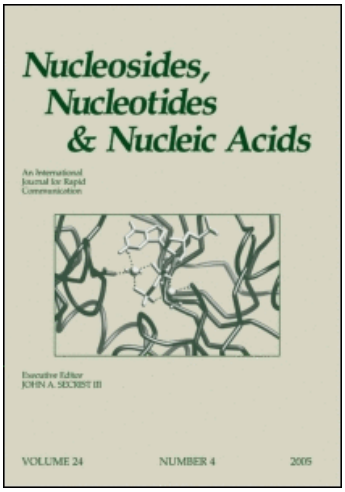

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Jarkko Roivainen[a]; Tatiana Elizarova[a]; Seppo Lapinjoki[a]; Igor A. Mikhailopulo[a]; Roman S. Esipov[b]; Anatoly I. Miroshnikov[b]

[a] Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland [b] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

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AN ENZYMATIC TRANSGLYCOSYLATION OF PURINE BASES

Jarkko Roivainen, Tatiana Elizarova, Seppo Lapinjoki, and Igor A. Mikhailopulo □ *Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland*

Roman S. Esipov and Anatoly I. Miroshnikov □ *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

□ *An enzymatic transglycosylation of purine heterocyclic bases employing readily available natural nucleosides or sugar-modified nucleosides as donors of the pentofuranose fragment and recombinant nucleoside phosphorylases as biocatalysts has been investigated. An efficient enzymatic method is suggested for the synthesis of purine nucleosides containing diverse substituents at the C6 and C2 carbon atoms. The glycosylation of N^6-benzoyladenine and N^2-acetylguanine and its O^6-derivatives is not accompanied by deacylation of bases.*

Keywords Base- and sugar-modified purine nucleosides; enzymatic synthesis; recombinant nucleoside phosphorylases

INTRODUCTION

It was shown that the reaction of an enzymatic transglycosylation offers obvious advantages over chemical methods.[1] Two approaches are successfully employed for the pentofuranosyl-transfer reactions catalyzed by nucleoside phosphorylases: one of these is based on the use of the whole bacterial cells displaying the uridine phosphorylase (UP) and/or thymidine phosphorylase (TP) and purine nucleoside phosphorylase (PNP) activities as a biocatalyst, the other makes use of the purified enzymes or cloned enzymes and overexpressed in bacteria. On the whole, both approaches mutually complement one another allowing to find out the most efficient way toward the desired nucleoside.

In the present communication, we have investigated an enzymatic transglycosylation of purine bases employing readily available pyrimidine or purine nucleosides as donors of the carbohydrate moiety, purine bases as

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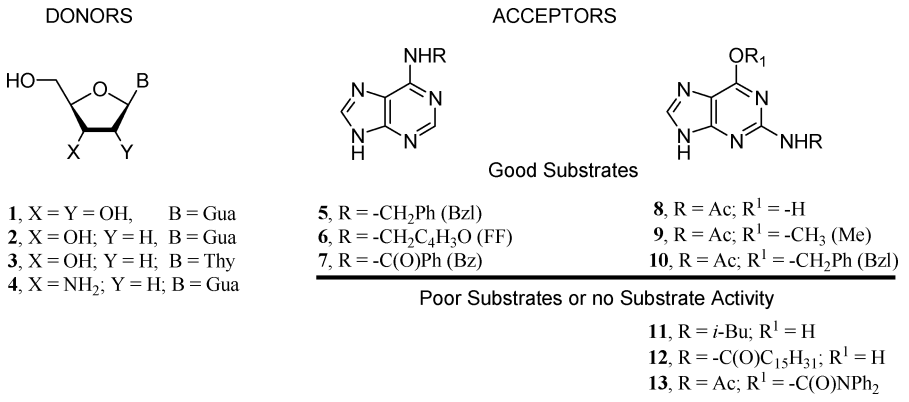
Address correspondence to I. A. Mikhailopulo, Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. E-mail: Igor.Mikhailopulo@uku.fi

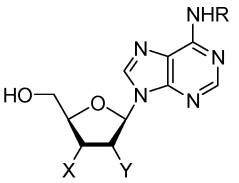


SCHEME 1 Structure of donors of a pentofuranose residue and purine heterocyclic bases investigated as acceptors in the reaction of an enzymatic transglycosylation.

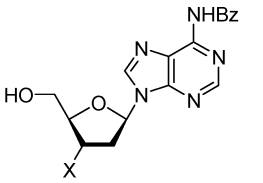
acceptors and the purified recombinant thymidine (TP) and/or purine (PNP) nucleoside phosphorylases[2] as biocatalysts.

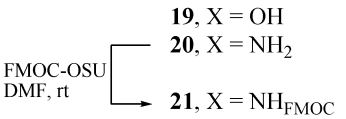
RESULTS AND DISCUSSION

Structure of donors and acceptor used in this study are shown in Scheme 1. The ribosylation, 2-deoxyribosylation and 3-amino-2,3-dideoxyribosylation of a number of purine heterocyclic bases was investigated employing commercially available guanosine (**1**), 2′-deoxyguanosine (**2**) and thymidine (**3**) as well as previously described 3′-amino-2′,3′-dideoxyguanosine (**4**)[3] as donors of the corresponding sugar moieties and either PNP or TP, or both enzymes simultaneously as biocatalysts.



Donor	Acceptor	Biocatalysts	Product No.	R	X	Y
1	**5**	PNP	**14**	Bzl	OH	OH
1	**6**	PNP	**15**	FF	OH	OH
3	**5**	TP & PNP	**16**	Bzl	OH	H
3	**6**	TP & PNP	**17**	FF	OH	H
4	**5**	PNP	**18**	Bzl	NH_2	H

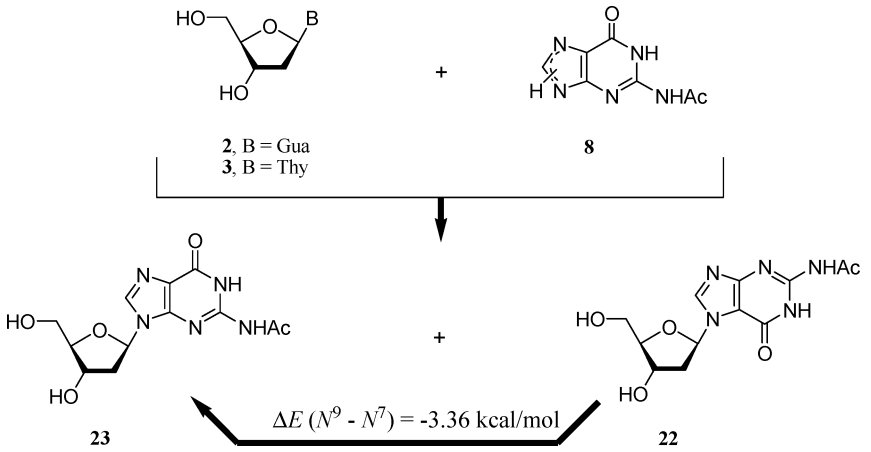




SCHEME 2 Structure of nucleosides **14-21**.

Recently, ribosides of N^6-benzyladenine (**5**) and kinetin (**6**) attract much attention as regulators that play an important role in the differentiation and development of human normal and malignant cells and might be clinically useful for treating some cancers.[4,5] Base halogenated derivatives of N^6-benzyladenine as well as sugar modified derivatives of cytokinines represent very important family of agonists and antagonists for adenosine receptors and are under intense all-round investigation.[6]

Biocatalytic ribosylation of N^6-benzyladenine (**5**) and kinetin (**6**) has been described using the whole *E. coli* cells as a biocatalyst and inosine as a donor of the ribofuranose residue.[7,8] In the present study, we investigated the ribosylation, 2-deoxyribosylation and 3-amino-2,3-dideoxyribosylation of both N^6-substituted purine bases using the respective nucleosides **1**, **2**, or **3** as donors of the sugar moiety and either PNP or TP, or both enzymes simultaneously as biocatalysts.[9] It was found that both N^6-benzyladenine (**5**) and kinetin (**6**) are good acceptors of the pentofuranose moieties studied and the desired nucleosides **14–18** have been obtained in 40–88% isolated yields (Scheme 2). Spatial arrangements of N^6-benzyladenine (**5**) and N^6-benzoyladenine (*7*) are similar. Analysis of their electronic structures (Hypercube, Inc., 2002; release 7.1; ab initio calculations, 6-31G*) also displayed a close similarity of both heterocycles especially the charges of N^7- (−0.264 e and −0.267 e, respectively) and N^9-nitrogens (-0.323 e and -0.322 e, respectively) that are involved in binding into the active center and coupling with a pentofuranose (see below). Indeed, N^6-benzoyladenine (**7**) was found to be an excellent substrate of PNP, and 2′-deoxyribosides **19** [donor 2′-deoxyguanosine (**2**)] and **20** (donor compound **4**) have been isolated from the reaction mixtures in 85 and 82% yields. Treatment of the latter with FMOC-OSU/DMF gave the crystalline FMOC-derivative (80%) **21** with



SCHEME 3 An enzymatic trans-2-deoxyribosylation of N^2-acetylguanine (**8**).

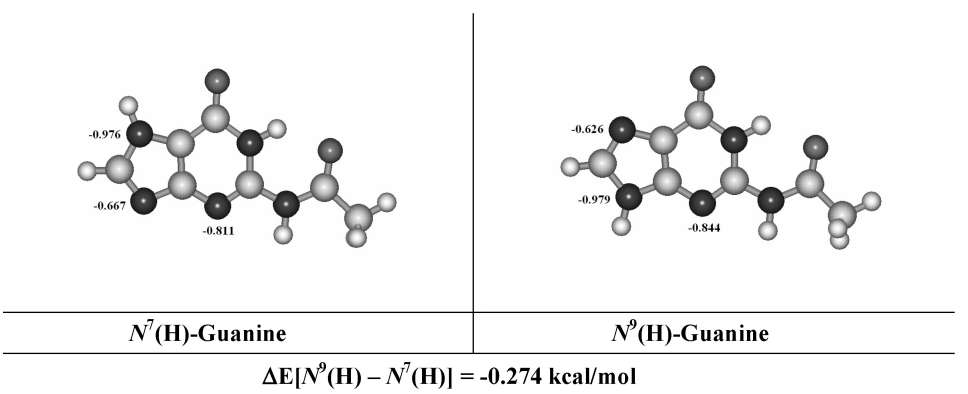


FIGURE 1 Computer-generated (ab initio, 6-31G*) electronic structures of isomeric N^2-acetyl-N^7(H) -and -N^9(H)-guanine.

an orthogonal protection of two amino functions that is very useful for the possible further applications of this compound (Scheme 2).

A number of N^2,O^6-substituted guanine derivatives **8–13** was investigated as substrates in the transglycosylation reaction using 2′-deoxyguanosine (**2**) or thymidine (**3**) as donors of the carbohydrate moiety. It was found that N^2-acetylguanine (**8**) is very good substrate for PNP and its 2-deoxy-D-ribosylation employing thymidine (**3**) as the pentofuranose donor and TP and PNP as biocatalysts gave rather interesting results (Scheme 3).

It was found that the N^7-isomer **22** predominantly forms at the beginning of the reaction along with the N^9-isomer **23**. The primarily formed N^7-isomer **22** is rearranged into the latter on prolonged heating of the reaction mixture that clearly points to the higher thermodynamic stability of the N^9-isomer **18**. Indeed, the ab initio calculations showed that the N^9-isomer **18** is thermodynamically favored over the N^7-isomer **17** by -3.36 kcal/mol. Interestingly, the ab initio calculations of isomeric N^7(H) and N^9(H) isomers of N^2-acetylguanine (**8**) manifested their thermodynamic similarity (Figure 1).

The use of thymidine as a donor (the ratio of **3**/**8** was 1.5:1.0, mol; standard reaction conditions) and TP and PNP as biocatalysts gave rise after 48 h to a ca. 1:6 mixture of N^7 -and N^9-isomers, from which the individual nucleosides **22** and **23** have been isolated in 9 and 55% yield, respectively. Under similar reaction conditions, the use 2′-deoxyguanosine as a donor and PNP as a biocatalyst led to a similar mixture, chromatographic separation of which gave individual nucleosides **22** (11%) and **23** (74%).

It was found that heterocyclic bases carrying out the bulky isobutyryl and palmitoyl substituents at the N^2-nitrogen, **11** and **12**, or diphenylcarbamoyl group at the O^6-oxygen **13** are not substrates for recombinant PNP as well as PNP within the whole *E. coli* cells. The formation of the corresponding nucleosides was not detected in the reaction mixtures.

On the contrary, N^2-acetyl-O^6-methylguanine (**9**) and N^2-acetyl-O^6-benzylguanine (**10**) have been found to be the good substrates for PNP and the respective N^9-2′-deoxy-β-D-ribofuranosyl nucleosides have been isolated in 95 and 50%, respectively. In both reactions, the formation of the N^7-isomers was not established by TLC and HPLC.

CONCLUSIONS

An efficient enzymatic method is suggested for the synthesis of purine nucleosides containing diverse substituents at C6 and C2 carbon atoms. N^2-Acetylguanine and N^2-acetyl-O^6-methyl- and -benzylguanine have been found to be good substrates of PNP. Initially, the former is predominantly glycosylated at the N^7-nitrogen and the N^7-glycoside formed is rearranged into thermodynamically more stable N^9-glycoside that is in accord with the ab initio calculations [$\Delta E(N^9 - N^7) = -3.36$ kcal/mol]. Steric control of the bulkiness of the N^2- and O^6-guanine derivatives realized by PNP is outlined. These data allow predicting the substrate properties of new purine analogues, which can be employed for an enzymatic transglycosylation aiming at the preparation of new biologically active compounds.

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9. *Standard reaction conditions*: the donor-acceptor ratio was 1.5–3:1 (mol); 5 mM *K*-phosphate buffer (pH 7.0); heterogeneous reaction mixture was gentle stirred at 50 °C monitoring the progress of reaction by TLC and HPLC; after the reaction reached constant equilibrium, SiO_2 was added and the mixture was evaporated to dryness, residue co-evaporated twice with abs EtOH, and SiO_2 with products was placed on the top of a column packed with SiO_2 in a mixture of CH_2Cl_2-MeOH (95:5, vol). Elution with a linear gradient of MeOH in CH_2Cl_2-MeOH (95:5, vol) gave the desired nucleosides.