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Chemoenzymatic preparation of nucleosides from furanoses

Marisa Taverna-Porro [a], Leon A. Bouvier [b], Claudio A. Pereira [b], Javier M. Montserrat [a,c,*], Adolfo M. Iribarren [a,d]

[a] *INGEBI (CONICET), Vuelta de Obligado 2490-(1428), Buenos Aires, Argentina*
[b] *Instituto de Investigaciones Médicas Alfredo Lanari (CONICET-UBA), Av. Combatientes de Malvinas 3150 (1427), Cap. Fed., Argentina*
[c] *Instituto de Ciencias, Universidad Nacional de Gral. Sarmiento, J. M. Gutierrez 1150, Los Polvorines (B1613GSX), Prov. de Bs. As., Argentina*
[d] *Laboratorio de Biotransformaciones, Universidad Nacional de Quilmes, Roque Saenz Peña 352 (1876) Bernal, Prov. de Bs. As., Argentina*

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Abstract

Chemoenzymatic preparation of ribose, deoxyribose and arabinose 5-phosphates was accomplished. These compounds were tested as starting materials in the enzymatic preparation of natural and modified purine and pyrimidine nucleosides, using an overexpressed *Escherichia coli* phosphopentomutase.

1. Introduction

The synthesis of modified nucleosides has an important impact in human health since many antiviral and antitumoral agents belong to this type of compounds.[1]

Traditionally, nucleoside analogues have been chemically prepared,[2] which often requires difficult and time consuming multistep processes, including selective protection–deprotection reactions. In particular, the stereoselective glycosylation of the pentose moiety is usually a challenging step.[3]

An alternative to chemical synthesis is the biotechnological preparation of nucleosides. This approach presents some advantages, usually the reaction conditions are smoother, environmentally friendly and principally regio- and stereoselective.[4] Along the last two decades, enzymatic protection–deprotection schemes[5] as well as biocatalyzed glycosylation have been reported.[6] Related to this last reaction, nucleoside phosphorylases (NPs) have been extensively used in the preparation of purine nucleoside from pyrimidine ones (transglycosylation).[7] The reaction proceeds via the formation of the corresponding α-furanose 1-phosphate, that is, subsequently used by the purine NPs (PNP) as substrate. This strategy has been applied to the preparation of sugar and base modified nucleosides, using isolated enzymes or whole cells.[8] A limitation of this approach, for sugar modified nucleosides, is that the corresponding pyrimidine analogue must be available. A way to overcome this problem is to employ furanose 1-phosphates as starting materials,[9] which unfortunately are limited by the synthetic difficulty and intrinsic unstability[10] of this class of compounds. Alternatively, furanose 1-phosphates can be enzymatically obtained from furanose 5-phosphates using phosphopentomutase (EC 5.4.2.7, PPM), an enzyme of the pentose pathway. PPM catalyses the reversible transfer of a phosphate group between the hydroxyls of positions 5 and 1 of ribose and deoxyribose, in bacteria and in mammal tissues. It showed to be a monomeric metalloenzyme,[11] Mn^{2+} dependent[12] and activated by glucose 1,6-diphosphate. PPM was originally overexpressed along with thymidine phosphorylase (TP) by Valentin-Hansen et al.,[13] but it was Wong[14] who first compared relative rates of overexpressed PPM catalysed conversion of D-pentose 5-phosphates to α-D-pentose 1-phosphates based on a coupled enzymatic assay with a PNP. In this work, they established that *Escherichia coli* PPM accepted as substrates

* Corresponding author. Tel.: +54 11 4783 2871; fax: +54 11 4786 8578.
E-mail address: jmonstser@ungs.edu.ar (J. M. Montserrat).

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deoxyribose 5-phosphate, ribose 5-phosphate and arabinose 5-phosphate but not 2,3-dideoxyribose 5-phosphate; unfortunately no indication of yields or absolute activities were reported. For the particular case of deoxynucleosides, a synthetic use of PPM has been reported by Ouerkerk et al.[15] for the preparation of ^{15}N and ^{13}C radiolabelled thymidine and 2′-deoxyuridine. In the same sense, Shimizu group[16] developed a one-pot microbial synthesis of 2′-deoxyribonucleoside in three steps.

Taking into account these antecedents, we decided to explore the chemoenzymatic production of ribose-, deoxyribose- and arabinose 5-phosphate, in order to evaluate the production of natural and modified nucleosides by the use of an overexpressed PPM, emphasizing on the generality of the method.

2. Results and discussion

In an attempt to develop a general strategy for the preparation of furanose 5-phosphates starting from ribose, arabinose and 2-deoxyribose (**1a**, **b**, **c**, Fig. 1), the corresponding methyl glycosides were prepared. While methyl riboside and methyl arabinoside were obtained mainly in the furanoside form, the methyl deoxyriboside was obtained as a 1:0.75 mixture of the pyranoside and furanoside forms that could not be separated. To overcome this problem, deoxyribose was locked in the furanose form using a different strategy that involved the regioselective acetylation of the 5-position using acetic anhydride and *Candida Antartica* lipase (CAL B) as biocatalyst.[17] Then, chemical acetylation using acetic anhydride in pyridine was performed to protect positions 1 and 3, obtaining peracetylated deoxyribose exclusively in the furanose form in 82% total yield starting from **1c**. Methyl riboside and methyl arabinoside were also peracetylated using previously described conditions.[18]

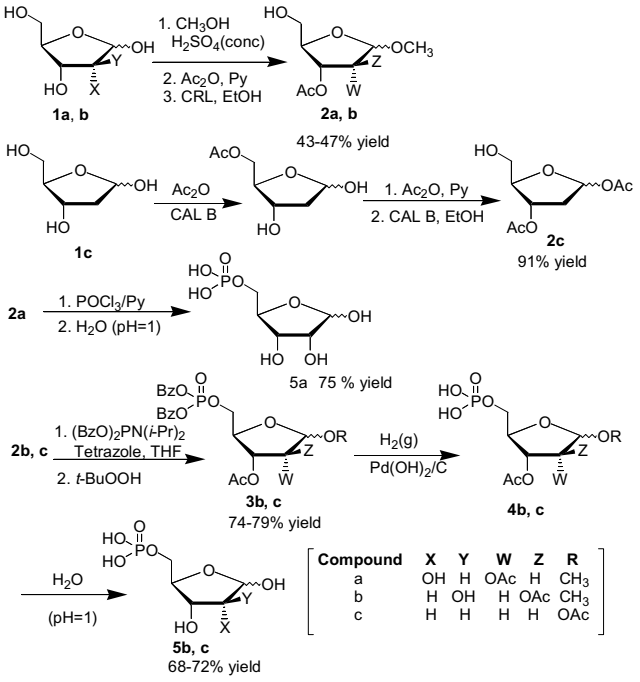


Fig. 1. Preparation of furanose 5-phosphates.

Then, the 5-acetyl groups of methyl 2,3,5-tri-*O*-acetyl-α,β-D-riboside, methyl 2,3,5-tri-*O*-acetyl-α,β-D-arabinoside and 1,3,5-tri-*O*-acetyl-2-deoxy-α,β-D-ribofuranose were regioselectively removed using lipase from *Candida rugosa* (CRL) or *Candida antartica* B lipase (CAL B) under alcoholysis conditions[19] to obtain products **2a**, **2b** and **2c** (Fig. 1).

Methyl 2,3-di-*O*-acetyl-α,β-D-riboside (**2a**, Fig. 1) was phosphorylated with phosphorous oxychloride in acetonitrile and pyridine. Total deprotection was achieved by the simple addition of water to the reaction medium. After neutralisation, inorganic phosphate was precipitated with aqueous $BaCl_2$ and filtered. The subsequent addition of ethanol to the solution afforded **5a** (Fig. 1) as its barium salt precipitate. This solid was transformed to the sodium form using ion exchange chromatography (Dowex 50WX2-200 (H)) in 75% total yield.

When the previously described reaction was applied to compounds **2b** and **2c**, the separation of inorganic phosphate from furanose 5-phosphate was not possible. As consequence that inorganic phosphate could cause PPM inhibition, a different phosphorylation strategy was assessed. Compounds **2b** and **2c** were phosphorylated using dibenzyl *N*,*N*-diisopropylphosphoramidite in THF with tetrazole activation and directly oxidised to phosphate by the action of *tert*-butyl hydroperoxide (Fig. 1). Phosphates **3b** and **3c** were purified by column chromatography and further debenzylated by hydrogenolysis catalysed with palladium hydroxide (**4b**, **4c**, Fig. 1). Total deprotection was achieved by chemical acid hydrolysis and the furanose 5-phosphates were precipitated as barium salts, isolated by centrifugation and transformed to the corresponding sodium salts using ion exchange chromatography. In this way, arabinose 5-phosphate (**5b**, Fig. 1) and 2-deoxyribose 5-phosphate (**5c**, Fig. 1) were obtained as pure products.

In order to determine the better conditions for the PPM catalysed reaction, the dependence of enzyme activity and reaction yield on pH, temperature, glucose 1,6-diphosphate, β-mercaptoethanol and phosphate concentrations was studied. For this purpose, the conversion of ribose 5-phosphate to adenosine, catalysed by the overexpressed PPM and commercial PNP, was selected as the model system. The enzyme activity and yield results are presented at Figure 2.

Recombinant PPM was further tested applying the optimised conditions previously discussed (Fig. 2), coupled to two different commercial NPs, PNP and TP. Although the commercial supplier (Sigma–Aldrich) indicated that the origin of the TP was *E. coli*, no data about the bacterial origin of PNP was available. The estimation of its molecular weight by gel electrophoresis (data not shown) and the preference for hypoxanthine over adenine (Table in Fig. 3) suggest that it belongs to the low molecular mass trimeric PNP family.[6]

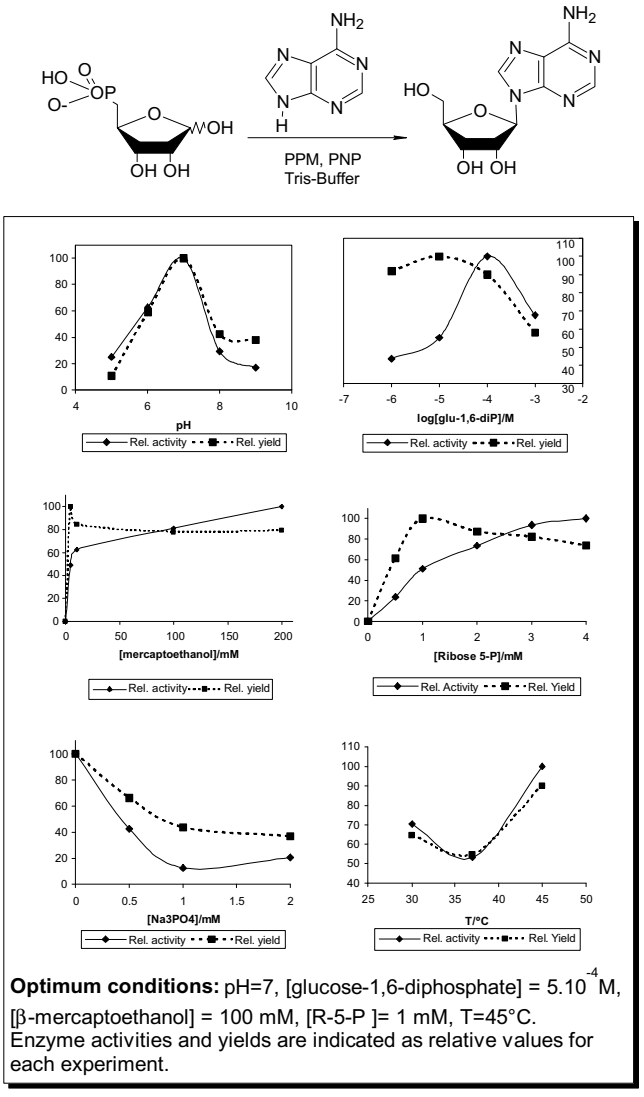
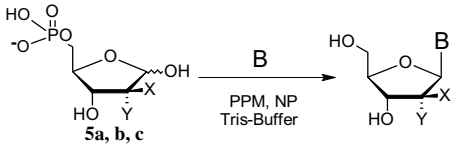


Fig. 2. Optimisation of PPM activity and yield using ribose 5-phosphate and adenine as model.

Using the previously synthesised ribose 5-phosphate (**5a**, Fig. 1), a set of natural and modified nucleosides were obtained using PPM and PNP as biocatalysts. Hypoxanthine was rapidly converted to inosine (2 h, **6**, Fig. 3) in quantitative yield. When adenine was used, the relative activity was lower (compound **7**, Fig. 3) but high reaction yields were achieved at longer reaction times. Then, a set of different substituted purines: 6-mercaptopurine, 6-chloro-2-aminopurine and 2-fluoropurine were assayed. Although activity and yield were acceptable for the preparation of the 6-mercaptopurine ribonucleoside (**8**, Fig. 3), results were not so positive in the case of 6-chloro-2-aminopurine ribonucleoside (**9**, Fig. 3) and 2-fluoroadenosine (**10**, Fig. 3). An interesting example was the synthesis of Ribavirine (Virazole) from **5a** and 1,2,4-triazole-3-carboxamide (**11**, Fig. 3). The reaction proceeded quantitatively and with high activity in 1.5 h.

Then, arabinose 5-phosphate (**5b**, Fig. 1) was used as a substrate of PPM and bases such as hypoxanthine, adenine, 6-mercaptopurine and 1,2,4-triazole-3-carboxamide were employed for the coupled glycosylation catalysed by the commercial PNP (**12**–**15**, Fig. 3). It has been previously established that arabinosides are poorer substrates than ribosides for PNP,[20] which may explain the low relative activities and yields obtained. The best result (46% yield) was achieved for compound **12**.



Comp. Nº	X	Y	NP	B	Rel. Act.	Yield/ %	Time/ h
6	H	OH	PNP	Hyp	100[a]	98	2
7	H	OH	PNP	A	14	90	48
8	H	OH	PNP	6-SH-Pu	66	89	2
9	H	OH	PNP	6-Cl-2-NH_2-Pu	1	25	32
10	H	OH	PNP	2-F-Ad	4.1	40	96
11	H	OH	PNP	TCA	127	100	1.5
12	OH	H	PNP	Hyp	1.15	46	46
13	OH	H	PNP	A	0.54	8	96
14	OH	H	PNP	6-SH-Pu	0.34	7	24
15	OH	H	PNP	TCA	0.27	19	72
16	H	H	TP	T	204	75	2
17	H	H	TP	U	148	74	2
18	H	H	TP	5-F-U	145	60	2.25
19	H	H	TP	5-Br-U	104	100	0.75

[a] The relative activity value of 100 % correspond to 0.49 μmol.min^{-1}.mg prot^{-1}

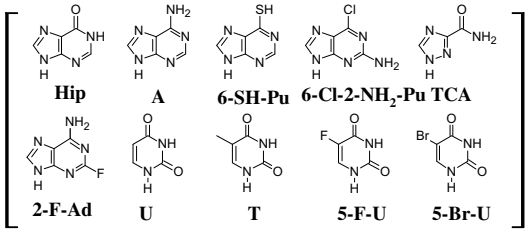


Fig. 3. Glycosylation reaction with different furanoses 5-phosphates and bases.

Finally, deoxyribose 5-phosphate (**5c**, Fig. 1) was used in combination with PPM and TP employing thymine, uracil, 5-fluorouracil and 5-bromouracil as bases (**16**–**19**, Fig. 3). In all cases, high activities and yields were observed. In the case of the preparation of compound **19**, a chemical dehalogenation produced by the β-mercaptoethanol present in the reaction medium was observed. For this particular case, an activity and yield optimisation experiment was performed against β-mercaptoethanol concentration, setting this value around 50 mM.

In this work, the preparation conditions for the chemoenzymatic obtainment of arabino, ribo and deoxy nucleosides have been explored, using different pyrimidine and purine bases. Experiments with diverse NPs sources and efforts to immobilise PPM are in progress.

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Supplementary data

Detailed experimental procedures for the protein expression and purification, synthesis of furanose 5-phosphates and analytical methods are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.087.

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