

## 2'-deoxyribosyltransferases: An efficient biocatalyst for the synthesis of nucleoside analogues

Fernández-Lucas J.<sup>\*1,2</sup>, Íria Mata Casar<sup>1</sup>, Alba Fresco-Taboada<sup>1</sup>, Carmen Acebal Sarabia<sup>1</sup>, Miguel Arroyo Sánchez<sup>1</sup>, Isabel de la Mata Riesco<sup>1</sup>

<sup>1</sup> Departamento de Bioquímica y Biología Molecular I, Universidad Complutense de Madrid (Spain)

<sup>2</sup> Departamento de Farmacia y Biotecnología, Universidad Europea de Madrid (Spain)

\* e-mail address: [jflucas@bbm1.ucm.es](mailto:jflucas@bbm1.ucm.es); [jesus.fernandez2@uem.es](mailto:jesus.fernandez2@uem.es)

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Nucleoside analogues have been traditionally synthesized by different chemical methods, which often require time-consuming multistep processes, including protection-deprotection reactions on the heterocyclic base and/or the pentose moiety, to allow the modification of natural occurring nucleosides (**1**). In this sense, enzymatic process for the synthesis of modified nucleosides is an interesting alternative to chemical procedures. This biotechnological approach shows many advantages such as very mild reaction conditions, high stereo and regioselectivity, and a friendly environmental technology (**2-4**). In general, nucleoside phosphorylases (NPs) and 2'-deoxyribosyltransferases (NDTs) have been used in nucleoside synthesis by mediating the transfer of glycosyl residues to acceptor bases. Such biotransformations have either been accomplished by employing soluble and immobilized enzymes or whole cells of microorganisms containing high percentages of the required enzyme

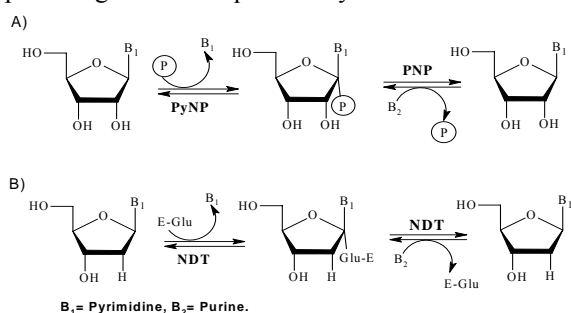


Fig. 1 – Nucleoside synthesis catalyzed by: A) Nucleoside phosphorylases. B) 2'-deoxyribosyltransferases

### RELEVANCE OF NUCLEOSIDE 2'-DEOXYRIBOSYL TRANSFERASES IN BIOCATALYSIS

Ribo and 2'-deoxyribosilnucleosides are formed by a purine or pyrimidine base bonded to the anomeric carbon of ribo or 2'-deoxyribose moiety. In this sense, both microbial nucleoside phosphorylases (NPs) and 2'-deoxyribosyltransferases (NDTs) can be used in

enzymatic nucleoside synthesis by mediating the transference of glycosyl residues to acceptor bases.

Nucleoside 2'-deoxyribosyltransferases (NDTs) catalyze the exchange between the purine or pyrimidine base of 2'-deoxyribonucleosides and free purine or pyrimidine bases (**5**). Reaction proceeds through intermediate formation of a covalently bound 2-deoxy- $\alpha$ -D-ribofuranosyl moiety, where the glycosidic hydroxyl of 2'-deoxyribose is esterified by a glutamic acid of the active site of NDTs (**Figure 1**). These enzymes are specific for 2'-deoxyribonucleosides, regioselective (N-1 glycosylation in pyrimidine and N-9 in purine), as well as stereoselective ( $\beta$ -anomers are exclusively formed) (**6**).

Nucleoside 2'-deoxyribosyltransferases are classified into two classes depending on their substrate specificity: NDT type I (PDT) specific for purines (Pur  $\leftrightarrow$  Pur) and NDT type II (NDT), which catalyzes the transfer between purines and/or pyrimidines (Pur  $\leftrightarrow$  Pur, Pur  $\leftrightarrow$  Pyr, Pyr  $\leftrightarrow$  Pyr) (**5-7**).

In this sense, the use of NDTs instead of NPs as biocatalysts for the synthesis of nucleoside analogues shows several advantages and drawbacks. Since thermodynamic equilibrium for PNP, but not for PyNP, is shifted towards nucleoside synthesis, most frequently exploited scheme of transglycosylation reaction consists in the combination of both PNP-PyNP or PNP-UP to synthesize purine nucleosides using pyrimidine nucleosides as donors and purine heterocyclic bases as acceptors (*one-pot, two-step* synthesis) (**Figure 1**).

On the other hand, a *one-pot, one-step* synthesis can be carried out by nucleoside 2'-deoxyribosyltransferases since NDT type II enzymes recognize both purine and pyrimidine 2'-deoxyribonucleosides. Therefore, transglycosylation reactions catalyzed by NDTs are more advantageous than those based on nucleoside phosphorylases, which need the performance of both enzymes. From an industrial point of view, immobilization of enzymes is essential to scale-up the process of nucleoside synthesis in order to become profitable. In the case of NPs, coimmobilization of both

enzymes (PNP-PyNP or PNP-UP) is needed (**8-9**), resulting in a heterogeneous biocatalyst, which leads to a less effective immobilization process and increase the final cost of product. On the contrary, immobilization of NDTs is more effective since only one enzyme is immobilized, so homogeneity of biocatalyst is 100 % (**11-14**). Taking into account such crucial differences, immobilized NDTs should be preferentially used in the industrial synthesis of natural and non-natural nucleosides

The aim of this work is show different aspects of the application of immobilized *Lr*NDT in non-natural nucleoside synthesis. In this sense the immobilization of *Lr*NDT has been performed in both Sepabeads EC-EP 303 (*SLr*NDT) and magnetic chitosan beads (*MCLr*NDT) in order to design a bioreactor for industrial synthesis of non-natural nucleosides. Functional characterization of these derivatives has been performed including the study of the effect of different pH and T conditions, as well as the effect of several water-miscible cosolvents on activity and stability of soluble and immobilized *Lr*NDT in order to design an optimal biocatalyst for the synthesis of non-natural nucleoside synthesis (Figure 2).

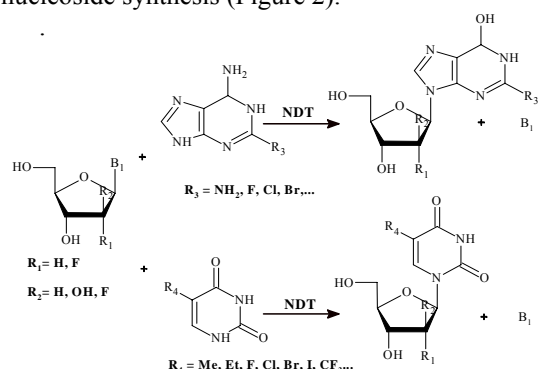


Fig. 2 – Nucleoside analogues synthesized by immobilized *Lr*NDT

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