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Opinion

# Enzymatic Platform for Nucleoside Analogues Production

**Sergi Pérez Ozcáriz***

*HealthBiotech General Coordinator in Drug Discovery Department, Inkemia IUCT Group, Álvarez de Castro, Mollet del Vallès, Barcelona, Spain*

***Corresponding Author:** Sergi Pérez Ozcáriz, HealthBiotech General Coordinator in Drug Discovery Department, Inkemia IUCT Group, Álvarez de Castro, Mollet del Vallès, Barcelona, Spain.

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One of the most interesting developments in the chemistry of nucleic acids has been the discovery of efficient synthesis pathways of nucleoside analogues [1]. These synthetic pathways allow the production of naturally occurring nucleosides as well as other containing modifications to the basic structure, the known as nucleoside analogues.

Nucleosides represent an important endogenous roll in many aspects as viral replication and cell division. Is for that reason that nucleoside analogues have been designed to interfere with these processes and to act as drugs [2-4]. The need to develop new nucleoside analogues and the great range of their therapeutic use has boosted many efforts in the optimization of their synthesis [5].

A wide variety of nucleoside analogues are currently developed to expand the range of action of these molecules as antiviral and anti-cancer agents and / or to modify the pharmacological and / or pharmacokinetic properties of these compounds [6].

One of the problems of nucleoside therapies is the acquisition of resistance and the side effects they present, such as cytotoxicity. The need for the development of new nucleoside analogs that present a greater effectiveness in long-term treatments [2] and the great applicability they present, has led to the investment of many efforts in the optimization of their synthesis, for which there are two approximations : Chemical synthesis and enzymatic synthesis [5].

## Chemical Synthesis

The chemical synthesis processes of nucleoside analogues present certain difficulties: they comprise multiple steps, they need several steps of protection and deprotection of reactive chemical groups of the heterocycle or the sugar in order to obtain the desired modification, they lack control of the anomeric center and little regioselective formation of the glycoside, as well as the need for the use of organic solvents that hinder the purification process and require a more complex waste management [7].

## Enzymatic Synthesis

The use of enzymes for nucleoside synthesis has several advantages because they have a high stereospecificity and regioselectivity and the reaction conditions are milder [8]. For example, glycosyltransferase reactions give exclusively the β-nucleoside anomer and the heterocyclic base is only glycosylated at one point [9]. Moreover, in many cases the starting nucleosides of the reactions are cheap, so that with this methodology higher value-added products and broad therapeutic applications can be obtained. The properties of the enzymes can serve to replace chemical processes in use or in combination with these, to improve existing methods.

For the synthesis of nucleoside analogues, there are two main classes of enzymes that catalyze the transfer of glycosidic residues from a starting nucleoside to an incoming nitrogen base: nucleoside phosphorylases and N-2'-deoxyribosyltransferases.

Nucleoside phosphorylases (NP) catalyze the reversible phosphorolysis of ribo- or deoxyribonucleosides giving ribose- or deoxyribose-1-phosphate and a purine or pyrimidine base. The addition of an acceptor nitrogen base allows the formation of a new nucleoside. In this reaction the equilibrium is displaced to the formation of nucleosides. There are quite a few types of NPs and their classification

is somewhat complex. This is because its presence is widely distributed in all groups of living organisms, and therefore there is great diversity in structure, affinity for substrates and stability under certain conditions. But they can mainly be classified into two large groups, the Purine Nucleoside Phosphorylase (PNP type) and the Pyrimidine Nucleoside Phosphorylase (PyNP type). Obviously, the difference between them is the type of nucleoside on which they act, whether it is a pyrimidine or purine type. A PNP is able to take a natural purpuric nucleoside and, in a "one-pot" reaction, change the base to another peckey that is present in the reaction medium. If this incoming (or acceptor) base is an unnatural base, the result will be a nucleoside analog, produced under mild and simple conditions. The same type of reaction can be explained by using PyNP-like enzymes with pyrimidine bases. But these types of reactions have limitations, among others the fact that they are equilibria, so the maximum reaction yield will hardly be high, for this we have to optimize enough the engineering of reaction. An alternative is to use two enzymes simultaneously, one type PNP and another type PyNP, to achieve, if the concentrations of substrates and enzymes are properly adjusted, to shift the equilibrium towards the formation of the desired products. For example, the combined use of these two types of enzymes to depart from a purine substrate and synthesize a pyrimidine. If, in addition, the use of a starting nucleoside whose base is very insoluble in the reaction medium is achieved, the equilibrium will be displaced in the desired direction. Since the discovery of nucleoside phosphorylase and the general description of its mechanism of action, these enzymes have been widely used in the synthesis of nucleoside analogues. Purine nucleoside phosphorylase (E.C.2.4.2.2.) and pyrimidine nucleoside phosphorylase (E.C.2.4.2.1) have been isolated from mammals as well as from many bacterial species exhibiting affinity for different substrates depending on the source from which they were obtained. For example, mammalian purine nucleoside phosphorylase does not accept adenosine as a natural substrate instead it can carry out the phosphorolysis of inosine and guanosine. However, this same enzyme but of Escherichia coli can accept all the purine nucleosides as a substrate [10].

Another interesting group of enzymes in this type of reactions is the group of NDTs. These enzymes catalyze the same type of transglycosylation reaction but without passing through the phosphate intermediate and without distinguishing between purine and pyrimidine bases / nucleosides. Its distribution in living beings is much smaller. However its functionality is somewhat more robust, accepting different types of substrates which expand the range of possible reactions to be performed.

A limitation of enzymatic approaches refers to the number (diversity) of nucleoside analogs obtainable by these methods. In the above-described enzymatic reactions to obtain, for example, sugar-modified nucleosides, the corresponding analogous purine / pyrimidine should be available. This can be solved if the 1-phosphate furanoses are used as the starting substrate in the reactions [11], but unfortunately the availability of this type of substrates is limited by difficulties in obtaining them and their intrinsic instabilities. As an alternative, it has been proposed to obtain 1-phosphate furanoses using phosphopentomutases (EC 5.4.2.7, PPM), an enzyme of the pentoses pathway.

Phosphopentomutases are metalloenzymes, $Mn^{2+}$ dependent [12], that catalyze the transfer of a phosphate group between hydroxyls of positions 5 and 1 of ribose and deoxyribose in bacteria and mammals [13].

In *Escherichia coli*, the phosphopentomutase is encoded by the deoB gene, the third gene of the deo operon, consisting of four structural genes (deoC, deoxyriboaldolase, deoA, thymidine phosphorylase, deoB and deoD, purine nucleoside phosphorylase). In *Bacillus subtilis* the phospho-hormone is encoded by the drm gene. Both sequences can be found in GenBank / EMBL Data Bank under accession numbers U14003 and U32685, respectively.

In the search for more universal and efficient approaches to the diversification of furanoses-1-phosphate usable as starting substrates in the synthesis of nucleoside analogs with nucleoside phosphorylases, some authors have suggested the coupling of another enzyme, ribokinase. (EC 2.7.1.15, RK). This enzyme catalyzes 5-O-phosphorylation of D-ribose in the presence of a phosphate group [14].

Ribokinases have been cloned from prokaryotic and eukaryotic sources and in all of them there is a high degree of similarity that places them in a single family of enzymes, called the PfkB family [15]. This family includes ribokinases, ketohexokinases, adenosine kinases, fructokinase, 2-dehydro-3-deoxygluconokinases, 1-phosphofructokinase and 6-phosphofructokinase.

Therefore, the use of the enzymes type PPM and RK has the function of providing a greater variety to the starting substrates for the

reactions NPs type. Namely, as discussed above, NP enzymes may incorporate a free base to a phosphate sugar in the 1'position. The problem is that these 1'-phosphate sugars are very unstable and are not commercial, so they have to be generated *in situ* by the same enzyme starting from a starting nucleoside which is phosphorylated. Commercial starting nucleosides are limited. Exchanging the base by a new acceptor base can generate a great variability of nucleoside analogues with modifications in the base, since it may have suitable chemical modifications before being incorporated. However, modifications in the sugars of the starting nucleosides are limited. If we can start with free sugars to incorporate a phosphate group in the 1'position and then add a base to them, the variety of products produced may be much larger, including more sugar modifications, which may not even be a pentose or until it is not even a sugar. PPM-like enzymes are able to take a 5'-phosphated sugar, which are stable and therefore commercial, and change this phosphate group from 5' to 1'. If this 1'-phosphated sugar is generated in the same reaction environment as a given NP and a base, nucleoside analogs can then be synthesized starting from a 5'- phosphate sugar and a base. Reducing the waste generated in this way, since there is a discarded donor base and the economic cost of the reaction, since the price of the raw material is lower. The RK fulfills the function of creating a 5'-sugar in the presence of Pi, so it goes further in supplying raw materials to the synthetic platform described. That is, it can generate in situ the 5'-phosphate molecules which the PPM subsequently converts to 1'-phosphate so that the NP in the presence of a base synthesizes the corresponding nucleoside analogue.

The combined and coordinated use of these four groups of enzymes provides a potent battery of possible reactions and considering that each group of enzymes has many representatives who differ among them in terms of their affinity for different substrates and their ability to accept non-natural substrates, a number of known or new nucleoside analogs can be generated that can be used to selectively combat different types of cancers or viral infections.

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