Batch and Flow Synthesis of Nucleosides by Enzymatic Transglycosylation

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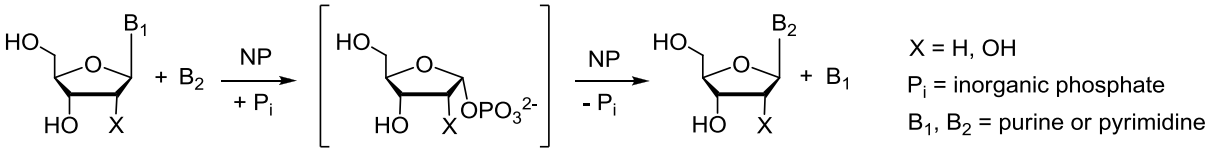
Enzymatic methods for the preparation of high-value products have clearly shown their potential in many areas, including nucleic acid chemistry. Enzymes of nucleic acid metabolism such as nucleoside phosphorylases (NPs, EC 2.4.2) can be conveniently used as biocatalysts in the synthesis of nucleoside analogues. These enzymes catalyze the reversible cleavage of the glycosidic bond of (deoxy)ribonucleosides in the presence of inorganic phosphate (P_i) to generate the nucleobase and α-D-(deoxy)ribose-1-phosphate (phosphorolysis). If a second nucleobase is added to the reaction medium, the formation of a new nucleoside can result (transglycosylation).

Because of its broad substrate specificity [1,2], a purine nucleoside phosphorylase from *Aeromonas hydrophila* (*Ah*PNP) was exploited to catalyze the "one-pot, one-enzyme" transglycosylation of 7-methylguanosine iodide with a series of 6-substituted purines, resulting in a moderate to high conversion (18-65%) of the bases into a 22-compound library of 6-substituted purine ribonucleosides [2]. Successively, *Ah*PNP was covalently immobilized [3,4] in a pre-packed column containing aminopropyl silica particles. The resulting *Ah*PNP-IMER (Immobilized Enzyme Reactor) was coupled on-line to a HPLC apparatus containing a semi-preparative chromatographic column. In such a system, "one-enzyme" transglycosylation and product purification were run in a single platform, affording a set of 6-modified purine ribonucleosides at a mg scale [4]. Using this "flow-based" approach, the synthesis of adenine nucleosides through a "two-enzyme" transglycosylation was carried out by connecting the *Ah*PNP-IMER to uridine phosphorylase from *Clostridium perfringens*, immobilized on a silica monolithic column (*Cp*UP-IMER).



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