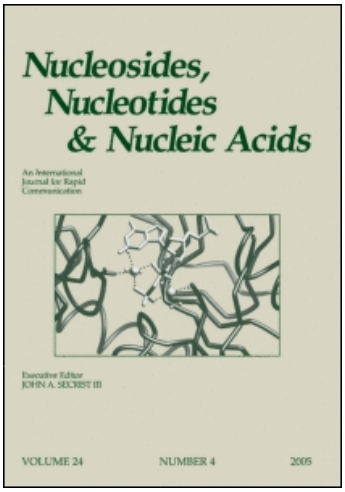

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Production of Nucleosides by Large-Scale Bioconversion

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PRODUCTION OF NUCLEOSIDES BY LARGE-SCALE BIOCONVERSION

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Abstract: the preparation of both natural and base modified ribonucleosides as well as 2'-deoxy, 2'-3'-dideoxy and arabinofuranosilnucleosides catalyzed by nucleoside phosphorylase enzymes is described. The catalyst consisted in *Enterobacter aerogenes* cells grown at high biomass and under phosphorylase inducing conditions. The implementation of this bioconversion approach in pilot scale was demonstrated by producing β-D-arabinofuranosyl-adenine (Ara-A) at multikilo levels.

Natural nucleosides and their modified analogs are precursors for a number of important antiviral and antitumor agents as well as for the preparation of oligonucleotides. Nucleosides can be obtained by multi-step chemical synthesis, even if this approach is plagued by low yields and by the formation of regio- and stereo-chemical isomers. Alternatively, nucleoside interconversion catalyzed by nucleoside phosphorylases has been described for the preparation of nucleosides and analogues[1,2], according to the following general scheme:

Pyrimidine-(deoxy)-ribo-nucleoside + inorganic phosphate
(Uridine-phosphorylase) ⇅
Pyrimidine base + (Deoxy)-ribose-1-phosphate

Purine base + (Deoxy)-ribose-1-phosphate
(Purine-nucleoside-phosphorylase) ⇅
Purine-(deoxy)-ribo-nucleoside + inorganic phosphate

where the coupling of uridine-phosphorylase (E.C.2.4.2.2) and purine-nucleoside-phosphorylase (E.C.2.4.2.1) efficiently transfers a sugar moiety from a donor nucleoside

to an acceptor heterocyclic base. The bioconversion approach has the potential to shorten the preparation processes by eliminating the need of protecting groups and to decrease production costs; moreover, since these reactions are carried out in water solutions environmental risks are greatly reduced. Despite the advantages of enzymatic transglycosylation, difficulties that limit the large-scale application of phosphorylase enzyme catalyzed bioconversion include the availability of convenient nucleoside sugar donors as well as efficient preparation of catalysts. These problems have been taken into consideration with the aim to set up a method for practical preparation of natural and modified nucleosides. Uridine, which can be considered a by-product of RNA hydrolysis, beside acting as ribose donor, was efficiently transformed by large-scale chemical reactions into 2'-deoxyuridine, 2'-3'-dideoxyuridine and β-D-arabinofuranosil-uracil (Ara-U) which respectively were used as donors of deoxyribose, dideoxyribose and arabinose moieties. High total and specific phosphorylase activities were obtained by growing *Enterobacter aerogenes* cells at high biomass and under phosphorylase inducing conditions. Bioconversion of 60 mM uridine or uridine derivatives in the presence of 20 mM appropriate base acceptors was applied to obtain a variety of natural 2'-deoxynucleosides (2'-deoxyguanosine, 2'-deoxyadenosine and thymidine) as well as 2'-3'-dideoxynucleosides (ddA) and modified analogues of pharmaceutical interest (2'-deoxyribofuranosil-2-6-dichloro-adenine, arabinofuranosil-2-6-diaminopurine, ribavirin and Ara-A) with yields ranging in most cases from 70% to 90%. The implementation of bioconversion at 1000 liter scale, starting from 40 mM Ara-U and 40 mM adenine, enabled to produce 5 kg/batch of β-D-arabinofuranosyl-adenine (Ara-A) with an overall yield of 50%.

In conclusion, we reported an efficient system for industrial preparation of both natural and modified nucleosides using uridine and its derived nucleosides as versatile starting sugar donors and *Enterobacter aerogenes* cells grown at high biomass as biocatalyst.

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