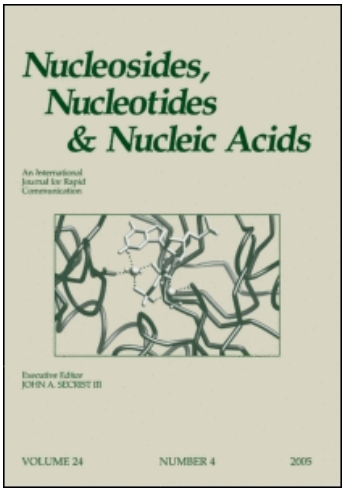

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Synthesis, Cytostatic Activity and Inhibition of Ribonucleotide Reductase by 5'-Phosphoramidates and 5'-Diphosphates, of 2'-*O*-Allyl-arabinofuranosyl Nucleosides

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SYNTHESIS, CYTOSTATIC ACTIVITY AND INHIBITION OF RIBONUCLEOTIDE REDUCTASE BY 5'-PHOSPHORAMIDATES AND 5'-DIPHOSPHATES, OF 2'-*O*-ALLYL-ARABINOFURANOSYL NUCLEOSIDES

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Abstract. The diphosphates of a series of 2'-*O*-allyl-1-β-D-arabinofuranosyl derivatives,previously obtained by us, have been prepared and tested for their inhibitory activity in an *in vitro* assay using R1 and R2 subunits of the purified recombinant mouse ribonucleotide reductase (RNR). 2'-*O*-Allyl-araU diphosphate proved to be inhibitory, with an IC_{50} of 100 μM. The 5'-phosphoramidate pronucleotide of 2'-*O*-allyl-araU was also prepared and tested for inhibition of tumor cell proliferation.

Ribonucleotide reductase (RNR) is an essential enzyme in DNA synthesis[1]. Because RNR is the rate limiting enzyme of deoxyribonucleoside diphosphate (dNDP) synthesis in eukariotes it is considered as an excellent target for antitumor/antiviral chemotherapy. Continuing our studies on RNR mechanism-based inhibitors, we have now prepared the diphosphates (DP) of some 2'-*O*-allyl-1-β-D-arabinofuranosyl nucleosides,[2] and evaluated their inhibitory activity against recombinant mouse RNR. In contrast with the lack of anti-tumor cell proliferative activity observed for the compounds *in vitro,*[2] under the condition tested 2'-*O*-allyl-araUDP proved to be inhibitory an IC_{50} of 100 μM, whereas 2'-*O*-allyl-araCDP was only marginally active (around 1 mM) and 2'-*O*-allyl-araADP completely inactive.

To reveal the basis of this discrepancy, the susceptibility of the parent nucleosides to phosphorylation by thymidine and 2'-deoxycytidine kinases have been investigated. The nucleoside analogs were very poor substrates of the kinases cited above, indicating that the poor phosphorylation, may represent a possible explanation of the lack of marked *in vitro* cytostatic activity. In an attempt to overcome this bottleneck we have prepared the prodrug of 2'-*O*-allyl-araU monophosphate, namely 2'-*O*-allyl-5'-(phenylethoxyalanylphosphate)-araU. However, when this latter compound was evaluated for its effect on cell proliferation it also proved inactive. The araU derivative was active on the isolated RNR but inactive against cell proliferation due to the lack of substrate activity for the kinases. Although the phosphoramidate approach does not necessarily guarantee a release of the monophosphate, absence of biological activity can also be explained by the lack of further phosphorylation of the drug to its 5'-diphosphate. Experimental procedures and further details will be reported in a full account.

Synthesis of nucleoside diphosphates. 2'-*O*-Allyl-araC 5'-diphosphate was obtained in 13% yield taking advantage of the procedure described by Kovacs and Otvos[3] for dNTPs. In the case of 2'-*O*-allyl-araU and -araA we adapted the procedure described by Poulter et al,[4] to obtain the expected diphosphates in 11% and 38% yield. **Synthesis of nucleoside phosphoramidates**. 2'-*O*-Allyl-5'-(phenylethoxyalanylphosphate)-araU pronucleotide was obtained, in 36% yield, following and adapting the procedure described by McGuigan et al.[5] Title compounds were characterized by ^{1}H and ^{31}P NMR, mass spectroscopy and HPLC.

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