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ON A CHEMOTHERAPEUTIC APPROACH USING NEUTRAL ANTI-HIV NUCLEOSIDE 5'-PHOSPHATES AS POTENTIAL PRODRUGS OF THEIR PARENT NUCLEOTIDES

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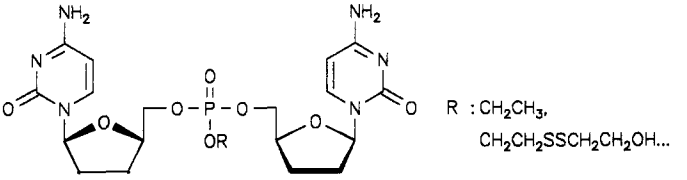
For a long time numerous uncharged phosphate derivatives of chemotherapeutic nucleosides as membrane-soluble prodrugs have been proposed in the literature in order to deliver following their intracellular hydrolysis the nucleoside 5'-monophosphates which will be further enzymatically phosphorylated to the active triphosphate forms.

According to this approach, we herein report our results on neutral bis (nucleosid-5'-yl) phosphates using dideoxycytidine (ddC) as a model (Figure). The rational behind these phosphate-modified dinucleotides is based on their facile transport through cell membranes, intracellular release of their phosphodiester followed by liberation of ddC and of its 5'-monophosphate (ddCMP) by action of phosphodiesterases.



Using the ISRP on line HPLC concept, we have determined the degradation kinetic parameters of these molecules in culture medium and identified their corresponding metabolites (bisddC phosphodiester, ddCMP, ddC).

We have found that the observed anti-HIV effect on MT4 and CEM cells could be directly correlated with the concentration of ddC liberated in culture medium under the incubation conditions (5 days at 37°C). Our results may also explain some recently reported biological data on nucleotide prodrugs, which have been attributed to an intracellular release of the nucleoside monophosphate and which actually would be the consequence of extracellular degradation to the free nucleoside.

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Anti-SV40 And Anti-HIV-$_1$ tat Activity Of Natural And Modified Oligonucleotides

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The use of antisense oligonucleotides in antiviral therapy is negatively influenced by the nuclease sensitivity of this class of molecules and by their intrinsically poor diffusion through the cell membrane. To circumvent these difficulties we have synthesized modified antisense sequences carrying L-2'-deoxynucleotides in the 5' and 3' termini. The effect of the oligonucleotides on SV40 replication was evaluated on VERO cells measuring the production of viral DNA. The anti-HIV-$_1$ activity was assessed on 293 cells constitutively expressing tat and CAT under control of the HIV LTR. The extent of inhibition was related to the amount of acetylated chloramphenicol produced. In both cases L-2'-deoxynucleotides resulted more active than their natural congeners, although they were not devoid of cytotoxicity. The oligonucleotide cell uptake exhibited the characteristics of an active process i.e. temperature dependence and intracellular concentrations much superior to the extracellular ones. Accumulation of L-oligomers was more than two fold higher than that of the natural derivatives, consistent with an increased resistance of the modified oligonucleotides towards 3'-5' exonucleases. Studies are in progress to verify if exonuclease-resistance can be obtained by inserting L-2'-deoxynucleotides at internal position of the oligomer sequence.