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Synthesis and Anti-HIV Evaluation of Some 5'-O-Phosphonomethyl-2',3'-Dideoxynucleosides

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5'-O-Phosphonomethylation of different pyrimidine 2',3'-dideoxyribosides was accomplished by reaction of the latter with diethyl p-toluenesulfonyloxymethane phosphonate in the presence of sodium hydride. The base-phosphonylated and sugar-phosphonylated derivatives could be readily distinguished by spectroscopic techniques. Protection of the uracil or thymine moiety with a N^3-benzoyl group failed to prevent base modification. However, O^4-methyl-protected 2',3'-dideoxyuridine readily afforded the 5'-O-phosphonylated derivative, which was then converted to both the 2',3'-dideoxyuridine and 2',3'-dideoxycytidine analogues. The 5'-O-phosphonomethyl derivatives of 3'-deoxythymidine, 2',3'-dideoxyuridine, 2',3'-dideoxycytidine, 3'-O-methylthymidine and 3'-amino-3'-deoxythymidine did not show an appreciable anti-HIV activity in MT-4 cells. In contrast, the 5'-O-phosphonomethyl derivatives of 3'-fluoro-3'-deoxythymidine and 3'-azido-3'-deoxythymidine achieved 50% inhibition of HIV-1 cytopathogenicity at a concentration of approximately 1 μM, which is 1000-fold higher than the concentration at which 3'-fluoro-3'-deoxythymidine inhibits HIV-1 replication in MT-4 cells. Also, the 5'-O-diphosphoryl derivative of 5'-O-phosphonomethyl-3'-deoxy-3'-fluorothymidine showed a 1000-fold lower affinity for HIV-1 reverse transcriptase than the 5'-triphosphate of 3'-deoxy-3'-fluorothymidine.

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Anti-HIV-1 Activity of Antiviral Compounds, as Measured by a Quantitative Focal Immunoassay in $CD4^+$ HeLa Cells and a Plaque Assay in MT-4 Cells

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Focus- and plaque-forming assays are often used in virological research to quantitate virus in biological samples. These techniques are known for their efficiency, simplicity, reproducibility, and reliability. The anti-human immunodeficiency virus type 1 (HIV-1) activities of selected nucleoside analogues and sulfated polysaccharides were evaluated by a plaque assay (PA) in MT-4 cells and a focal immunoassay (FIA) in $CD4^+$ HeLa cells. Similar 50% inhibitory concentrations (IC_{50}) were obtained for the sulfated polysaccharides when measured by PA or FIA: the IC_{50} values of dextran sulfate and pentosan polysulfate were 0.80 μg/ml and 0.35 μg/ml, respectively. Also, comparable IC_{50} values were obtained for the purine 2',3'-dideoxyribosides DDA, DDI and DDG, when evaluated by PA or FIA: their IC_{50} values ranged from 1.42 to 2.71 μM. In contrast, the IC_{50} values of the pyrimidine 2',3'-dideoxyribosides were invariably 4- to 10-fold lower when monitored by PA than if measured by FIA: IC_{50} of AZT, D4T and DDC, as based on PA, were 0.015 μM, 0.094 μM and 0.038 μM, respectively; their IC_{50} values, as based on FIA, were 0.062 μM, 0.285 μM and 0.463 μM, respectively. The differences in anti-HIV-1 activity found for AZT, D4T and DDC when evaluated in the PA and FIA assay systems may at least in part be related to differences in the metabolism (i.e. thymidine kinase and/or 2'-deoxycytidine kinase levels) between MT-4 and $CD4^+$ HeLa cells.