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Stereoselective Synthesis of 2' Fluorinated Nucleosides: A Novel Method for Stereoselective Introduction of Fluorine into Sugar Ring Precursors, J. McAtee and D. C. Liotta, Emory University, Department of Chemistry, Atlanta, GA, USA

Fluorinated nucleoside analogs represent an important class of chain terminators which are currently receiving much attention as potential anti-viral drugs. Fluorine is an especially important functionality for analogs of natural biomolecules. This is due to the enormous electronegativity of fluorine and its small steric size. For a negligible change in the size of a molecule, dramatic changes in the electronic properties of that molecule are realized when fluorine replaces a hydrogen. Of particular interest is the fluorination of the sugar ring of the nucleoside. This is usually dependent on the presence of hydroxyl groups in the sugar or anhydro nucleoside intermediates for stereoselective introduction of fluorine. Recently, we have developed a convenient method for the completely stereoselective introduction of fluorine into a chiral lactone intermediate through the use of the novel N - fluorobenzenesulfonamide reagent. The chiral lactone is in turn accessed by a short synthesis from readily available glutamic acid. This key fluorinated intermediate may then be transformed in three steps and in high yield into a nucleoside. A wide variety of pyrimidine bases (and possibly purine bases) may be utilized and several nucleosides have been synthesized in this laboratory using this methodology.

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Effects of ß-D-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine 5'-triphosphate (ß-D-D4FC-TP) and its ß-L-enantiomer 5'-triphosphate (ß-L-D4FC-TP) on viral DNA polymerases. A. Faraj[1], R.F. Schinazi[2], A. Joudawlkis2, Z. Lesnikowski[2], A. McMillan[2], C.D. Morrow[1] and J.-P. Sommadossi[1]. University of Alabama at Birmingham, Birmingham, AL, USA[1] and VA Medical Center/Emory University, Decatur, GA, USA[2].

ß-D-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine (ß-D-D4FC) and its optical isomer ß-L-2',3'-didehydro-2',3'-dideoxy-5-fluorocytidine (ß-L-D4FC) have potent and selective activity against human immunodeficiency virus and hepatitis B virus (HBV) in vitro. The ß-D-enantiomer is one of the most anti-HBV agent with an EC_{50} value of 0.003 µM in 2.2.15 cells and a therapeutic index of over 88,000. ß-L-D4FC was also active against HBV replication (EC_{50} = 0.01 µM) but it was significantly more toxic in various cell lines. The 5'-triphosphates of ß-D-D4FC and ß-L-D4FC were chemically synthesized and their inhibitory effects toward wild type (WT) HIV-1 reverse transcriptase (RT), mutant RT at position 184 (substitution of methionine to valine [M184V]) and woodchuck hepatitis virus (WHV) DNA polymerase were evaluated. In vitro kinetics studies demonstrated that ß-D-D4FC-TP and ß-L-D4FC-TP inhibited WT HIV-1 RT and WHV DNA polymerase with an IC_{50} values approximating 1 µM. Use of M184V HIV-1 RT resulted in significant increase in IC_{50} value for ß-L-D4FC-TP whereas the elevation in IC_{50} value of ß-D-D4FC-TP was moderate. By using sequencing analysis, ß-D-D4FC-TP exhibited potent DNA chain terminating activity toward both WT and mutant HIV-1 RT, whereas ß-L-D4FC-TP was only recognized by the parental RT. In effect, the ß-L-derivative selects for virus with a single mutation from M184 to V in the RT gene. Whereas ß-L-D4FC was cross-resistant with (-)-FTC and 3TC, the ß-D-enantiomer was not. These results emphasize the importance of evaluating each enantiomer as a unique entity and demonstrate that not all ß-L-enantiomers have superior biological profiles compared to their ß-D-counterparts (supported by NIH and VA).

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Use of Human Hepatocyte Primary Cultures to Evaluate the Cellular Pharmacology of Anti-hepatitis Agents.

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In vitro assessment of potential anti-hepatitis agents is routinely performed utilizing human hepatoma cell lines. Although these cells are well differentiated, their transformed state can result in abnormal drug metabolism and mechanisms of toxicity. Hence, transformed cells may not mimic the metabolic characteristics of normal adult human hepatocytes. Our laboratory has developed an in vitro model utilizing normal adult human hepatocytes in primary culture to elucidate the potential metabolic activation and deactivation pathways of several anti-hepatitis agents. Briefly, human hepatocytes are obtained from non-transplantable donor tissue and plated at a cell density of 0.8 X 10^6 cells per well on collagen coated 12-well dishes in William's E medium containing 10% FBS, 2 mM glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin and 10µg/ml insulin. Twelve hours after seeding, media is removed and replaced with serum-free media containing 1µM hydrocortisone, 10 µg/ml transferrin, 5 µM ethanolamine, 0.1 µg EGF, and 10 ng/ml selenium. The cells are further incubated for 12 h in this new media and then exposed to anti-hepatitis agents for 24 h. Cell monolayers are washed with ice-cold PBS, scraped off in 60% methanol and extracted overnight at -20°C. Extracts are dried, reconstituted in water and analyzed by HPLC. The culture media is also analyzed by HPLC. Using this system, the metabolic disposition of 3TC, ß-L-2',3'-dideoxy-5-fluorocytidine (L-FddC) and Famciclovir are currently under evaluation. Preliminary data suggest that the activation pathways for these agents are qualitatively similar to those observed in Hep G2 cells. Of particular importance will be to determine if triphosphate levels achieved in cultured hepatocytes are adequate for inhibition of viral replication and predictive of levels reached in vivo.

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IgM anti-preS2 monitoring during combined corticosteroid/interferon alpha2b therapy in chronic hepatitis B. S.Sylvan,GZ Fei, GB Yao,U.Hellström.Dept.Comm.Dis.Control, Sthlm,Sweden,Jing An Hosp,Shanghai,China

A direct binding ELISA was established for quantitative determination of serum IgM antibodies towards a synthetic peptide corresponding to a selected segment(14-21) of the preS2 gene product containing an immunodominant linear B-cell epitope.The prevalence of IgM a-preS2 antibody titers>1000 for HBeAg pos. patients with chronic HBV-infection was 38% and 10% for HBeAg neg. subjects.Recombinat interferon(IFN) alpha 2b with an antecedent short course of corticosteroids was administered in 8 Chinese patients with chronic HBV-infection.The IgM a-preS2 reactivity was consequitively monitored during treatment and patients were followed for more than 1 year.A close ass. between the presence of pretreatment IgM a-preS2 in serum and the capacity to respond favourable to the combined prednisone/IFN therapy was detected.The IgM a-preS2 titres decreased during treatment with subsequent loss of detectable antibodies 8-16 weeks after the initiation of therapy.This decrease was concomitant with an ALT augmentation preceeding the disappearance of HBV-DNA and HBe/a-HBe seroconversion.No long term remission was observed in treated patients who lacked detectable levels of pre treatment IgM a-preS2 in circulation. (Fei et al,J.Med.Virol. 46:138,1995)