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β-L-Thymidine and β-L-2'-deoxycytidine are potent, selective and specific anti-hepatitis B virus agents. A Faraj[1], AG Loi[2], L Cui[1], MY Xie[1], M Bryant[2], EG Bridges[2], A Juodawlkis[2], C Pierra[2], D Dukhan[2], G Gosselin[3], J-L Imbach[3], RF Schinazi[4], B Pai[4], B Korba[5], P Marion[6], and J-P Sommadossi[1]. [1]Univ. of Alabama at Birmingham, USA; [2]Novirio Pharmaceuticals; [3]Univ. de Montpellier II, France; [4]Emory Univ. and VA Med. Center, Decatur, GA, USA; [5]Georgetown Univ., Rockville, MD, USA; and [6]Stanford Univ., Stanford, CA, USA.

Two representatives of the β-L-2'-deoxynucleoside class of compounds, β-L-thymidine (L-dT, NV-02B) and β-L-2'-deoxycytidine (L-dC, NV-02C) inhibited HBV replication in 2.2.15 cells (EC_{50} 0.05-0.26 μM), and duck HBV in primary duck hepatocytes ($EC_{50} \leq 0.05$ μM). The combination of L-dT and L-dC was synergistic at near equimolar concentrations in 2.2.15 cells. L-dT and L-dC were non-cytotoxic (CC_{50} > 2000 μM) and did not inhibit growth of human bone marrow progenitor cells (CFU-GM and BFU-E, > 10 μM). Viral rebound in L-dT or L-dC treated 2.2.15 cells occurred after drug withdrawal but remained less than 50% through day 18 post-treatment. This was consistent with the extended intracellular half-lives of L-dTTP and L-dCTP. No antiviral activity was detected *in vitro* against other DNA or RNA viruses including HIV, RSV, HSV, VZV, HCMV, EBV, measles virus, adenovirus, rhinovirus, influenza or parainfluenza virus. The structure-activity relationship within this class of β-L-2'-deoxynucleosides showed that the 3'-hydroxyl group was required to differentiate inhibition of HBV from HIV or other antiviral activity. L-dTTP and L-dCTP inhibited woodchuck hepatitis virus DNA pol, with an IC_{50} of 0.24 and 1.8 μM, respectively, while neither was a substrate for the HIV RT. L-dTTP and L-dCTP were not substrates for human DNA polymerases α, β, or γ up to 100 μM. There was no reduction in mitochondrial DNA content, no lactic acid accumulation and no alteration in mitochondrial morphology or function up to 10 μM. L-dT and L-dC are attractive drug candidates for further development for the treatment of chronic HBV infection.

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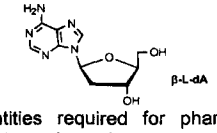
β-L-2'-Deoxynucleosides as Potent Anti-HBV Agents (Part I) : Large-Scale Stereospecific Synthesis of β-L-2'-Deoxyadenosine. D Dukhan,[1] C Pierra,[1] M Bryant,[2] JP Sommadossi,[3] J-L Imbach[1] and G Gosselin[1]. [1]Laboratoire Coopératif Novirio-CNRS-Université Montpellier II, France; [2]Novirio Pharmaceuticals; [3]University of Alabama at Birmingham, USA.

Recently there has been a growing interest in unnatural L-enantiomer nucleosides as antiviral agents.[1] We have discovered that the previously described β-L-2'-deoxyadenosine (β-L-dA)[2-4] exhibits potent, selective and specific activity against Human Hepatitis B Virus *in vitro* and *in vivo*.



To produce the quantities required for pharmacokinetic and toxicology studies in rats and monkeys, a novel synthetic route was selected and reduced to practice.

1) Wang, P.; Hong, J. H.; Cooperwood, J. S.; Chu, C. K. *Antiviral Res.* **1998**, *40*, 19-44.
2) Robins, M. J.; Khwaja, T. A.; Robins, R. K. *J. Org. Chem.* **1970**, *35*, 636-639.
3) Fujimori, S.; Iwanami, N.; Hashimoto, Y.; Shudo, K. *Nucleosides, Nucleotides* **1992**, 341-349.
4) Boudou, V.; Gosselin, G.; Imbach, J.-L. *Nucleosides, Nucleotides* **1999**, *18*, 607-609.

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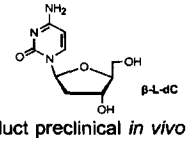
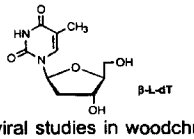
β-L-2'-Deoxynucleosides as Potent Anti-HBV Agents (Part II): Large-Scale Stereospecific Syntheses of β-L-2'-Deoxycytidine and β-L-Thymidine. C. Pierra,[1] D. Dukhan,[1] M. Bryant ,[2] J.-P. Sommadossi,[3] J.-L. Imbach[1] and G. Gosselin[1]. [1]Laboratoire Coopératif Novirio-CNRS-Université Montpellier II, France ; [2]Novirio Pharmaceuticals; [3]University of Alabama at Birmingham, USA.

During the last few years there has been a growing interest in unnatural L-enantiomer nucleosides as antiviral agents.[1] We have recently discovered that the previously identified β-L-2'-deoxycytidine (β-L-dC)[2-5] and β-L-thymidine (β-L-dT)[2-4,6] exhibit potent, selective and specific activity against hepadnaviruses *in vitro* and *in vivo*.





To conduct preclinical *in vivo* antiviral studies in woodchucks and pharmacokinetic and toxicology studies in rats and monkeys, large scale synthesis was required. We will describe the process chemistry of β-L-dC following the Holy's procedure[3] and two different strategies which have been developed to produce β-L-dT.

1) Wang, P.; Hong, J. H.; Cooperwood, J. S.; Chu, C. K. *Antiviral Res.* **1998**, *40*, 19-44.
2) Fujimori, S.; Iwanami, N.; Hashimoto, Y.; Shudo, K. *Nucleosides, Nucleotides* **1992**, *11*, 341-349.
3) Holy, A. *Collec. Czech. Chem. Commun.* **1972**, *37*, 4072-4087.
4) Urata, H.; Ogura, E.; Shinohara, K.; Ueda, Y.; Akagi, M. *Nucl. Acids Res.* **1992**, *20*, 3325-3332.
5) Lin, T.-S.; Luo, M.-Z.; Liu, M.-C. *Nucleosides, Nucleotides* **1994**, *13*, 1861-1870.
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Antiviral activity of β-L-thymidine and β-L-2'-deoxycytidine in the woodchuck model of chronic hepatitis B infection. EG Bridges[1], A Juodawlkis[1], A Faraj[2], B Tennant[3], B Korba[4], RF Schinazi[5], T Barnett[5], G Gosselin[6], J-L Imbach[6], C Pierra[1], D Dukhan[1], J-P Sommadossi[2], M Bryant[1]. [1]Novirio Pharmaceuticals; [2]Univ. of Alabama at Birmingham, USA; [3]Cornell Univ., Ithaca, NY; [4]Georgetown Univ., Rockville, MD, USA; [5]Emory Univ. and VA Med. Center, Decatur, GA, USA; [6]Univ. de Montpellier II, France

β-L-Thymidine (L-dT, NV-02B) and β-L-2'-deoxycytidine (L-dC, NV-02C) are representatives of the class of β-L-2'-deoxynucleosides which have specific anti-HBV activity. L-dT and L-dC were shown to be phosphorylated by thymidine kinase and 2'-deoxycytidine kinase in human and woodchuck liver extracts. L-dTTP and L-dCTP inhibited woodchuck hepatitis virus (WHV) DNA polymerase (IC_{50} = 0.24 and 1.8 μM, respectively). Antiviral activity and safety of L-dT and L-dC was investigated in a woodchuck model of chronic HBV infection. Woodchucks chronically infected with WHV (>10^{11} genome equiv/ml serum) were treated once daily with 0.01, 0.1, 1 and 10 mg/kg L-dT or L-dC (3 animals per group) for 28 days. Control animals received lamivudine (10 mg/kg/d) or vehicle alone. In the L-dC treated animals (10 mg/kg/d), viral load was reduced by as much as 6 logs by day 14 to 21. Viral rebound was seen within 1 week following cessation of treatment with L-dC. In the L-dT treated animals (10 mg/kg/d), viral load fell below the limit of detection of the dot blot assay following day 14. By day 14 to 28, viral load had dropped by as much as 8 logs from baseline by quantitative PCR assay. Post-treatment virus rebound in the L-dT treated animals was delayed 2-4 weeks. All animals gained weight and there was no evidence of drug-related toxicity during the 4-week treatment period or during 8 weeks of post-treatment follow-up. A twelve-week safety and antiviral activity study in chronically infected woodchucks is in progress. Development of L-dT and L-dC as anti-HBV compounds is warranted based on currently available results obtained using this animal model.