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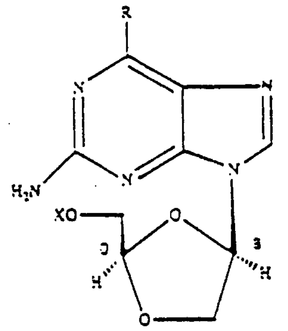
(57) Abstract

The invention is a method for the treatment of humans infected with HBV that includes administering an HBV treatment amount of an enatiomerically pure β-D-dioxolanyl purine nucleoside of formula (I), wherein R is OH, Cl, NH_2 or H, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt.



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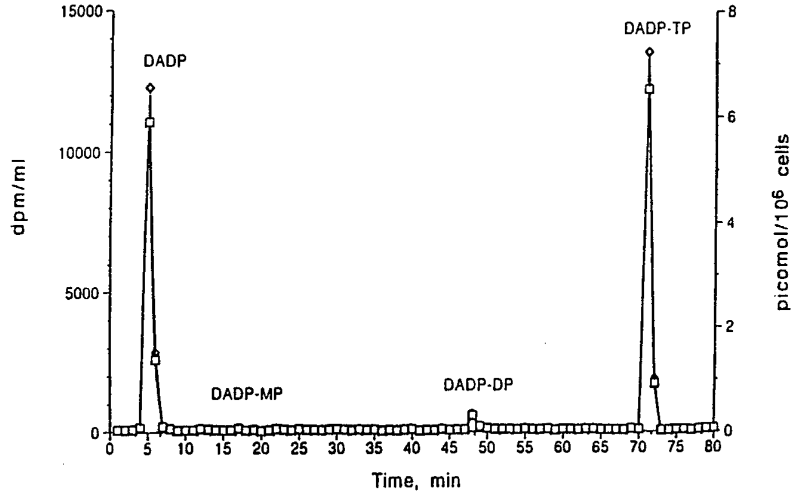

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ENANTIOMERICALLY PURE β-D-DIOXOLANE NUCLEOSIDES WITH SELECTIVE ANTI-HEPATITIS B VIRUS ACTIVITY

Background of the Invention

This invention is in the area of methods for the treatment of hepatitis B virus (also referred to as "HBV") that includes administering an effective amount of one or more of the active compounds disclosed herein, or a pharmaceutically acceptable derivative or prodrug of one of these compounds.

HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

Hepatitis B virus has reached epidemic levels worldwide. After a two to six month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed.

Patients typically recover from acute viral hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone, and worldwide, almost 300 million carriers. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer.

In western industrialized countries, high risk groups for HBV infection include those in contact with HBV carriers or their blood samples. The epidemiology of HBV is in fact very similar to that of acquired immunodeficiency syndrome, which accounts for why HBV infection is common among patients with AIDS or HIV-associated infections. However, HBV is more contagious than HIV.

A human serum-derived vaccine has been developed to immunize patients against HBV. Vaccines have been produced through genetic engineering. While the vaccine has been found effective, production of the vaccine is troublesome because the supply of human serum from chronic carriers is limited, and the purification procedure is long and expensive. Further, each batch of vaccine prepared from different serum must be tested in chimpanzees to ensure safety. In addition, the vaccine does not help the patients already infected with the virus.

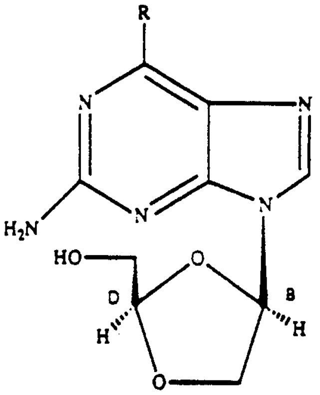
Daily treatments with α-interferon, a genetically engineered protein, has also shown promise. However, to date there is no known pharmaceutical agent that effectively inhibits the replication of the virus in humans.

In light of the fact that hepatitis B virus has reached epidemic levels worldwide, and has severe and often tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat humans infected with the virus that have low toxicity to the host.

Therefore, it is another object of the present invention to provide a method and composition for the treatment of human patients or other hosts infected with HBV.

Summary of the Invention

In a preferred embodiment, the invention is a method for the treatment of humans infected with HBV that includes administering an HBV treatment amount of an enantiomerically pure β-D-dioxolanyl purine nucleoside of the formula:



wherein R is OH, Cl, NH2. or H, or a pharmaceutically acceptable salt or derivative of the compound, optionally in a pharmaceutically acceptable carrier or diluent. The compound wherein R is chloro is referred to as (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-I,3-dioxolan-4-yl]purine. The compound wherein R is hydroxy is (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine. The compound wherein R is amino is (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine. The compound wherein R is hydrogen is (-)-(2R,4R)-2-amino-

9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine. The absolute configuration of these compounds has not been determined by crystallography. Designations are based on comparison of the structure to the configuration of the parent sugar used to make the compound. In another embodiment, an effective amount of the β-L-dioxolanyl purine nucleoside enantiomer, or a racemic mixture of the β-L- and β-D-dioxolanyl purine nucleoside is administered to the patient.

It has been discovered that the EC_{50} for ACPD ((-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine); and DAPD ((-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine)) for HBV DNA replication intermediates or HBV virion synthesis inhibition is close to 0.1 μM. No marked cytotoxicity was noted for DAPD, ACPD, or DG ((-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine) when tested up to 300 μM in 2.2.15 cells. These three purine nucleosides were significantly non-toxic to myeloid and erthroid cells in clonogenic assays (IC_{50} = 50 to greater than 100, as compared to AZT IC_{50} of 1 μM).

It has also been discovered that DG, DAPD, ACPD, and APD ((-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine) are not inhibitors of enzymes involved in purine and pyrimidine biosynthesis, such as adenosine deaminase, purine nucleoside phosphorylase, hypoxanthine-guanosine phosphoribosyl transferase, adenosine kinase, inosine kinase, cytidine kinase, xanthine oxidase, aldehyde oxidase and xanthine dehydrogenase, when tested at a concentration up to 1 mM.

The disclosed β-dioxolane purine nucleosides, or their pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been 5 exposed to HBV.

In one embodiment of the invention, one or more of the active compounds is administered in an alternative fashion with one or more other anti-HBV agents, to provide effective anti-HBV treatment. Examples of anti-HBV agents that can be used in alternation therapy include but are not limited to the enantismer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC", see W092/14743), its physiologically acceptable derivative, or physiologically acceptable salt; (-)-enantismer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (also referred to as "BCH-189" or 3TC, see EPA Publication No. 0 382 526), its physiologically acceptable derivative, or physiologically acceptable salt; an enantismer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU); carbovir, or interferon.

Any method of alternation can be used that provides treatment to the patient. Nonlimiting examples of alternation patterns include 1-6 weeks of administration of an effective amount of one agent followed by 1-6 weeks of administration of an effective amount of a second anti-HBV agent. The alternation schedule can include periods of no treatment.

In another embodiment, the active compound or its derivative or salt can be administered in combination with another anti-HBV agent, including those listed above. In general, during alternation therapy, an effective dosage of each anti-HBV agent is administered serially, whereas in combination therapy, a reduced dosage of two or more anti-HBV agents are administered together. The dosages will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

Brief Description of the Figures

Figure 1 is an illustration of the method of preparation of a variety of enantiomerically pure β-D-dioxolanyl purine nucleosides.

Figure 2 is a graph of the effect of purine dioxolanes and AZT on colony formation of human erythroid (BFU-E) precursor cells, as measured in terms of percent of cells of control versus the log of the concentration of test drug (AZT, 3'-azido-deoxy-thymidine; APD, (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1, 3-dioxolan-4-yl]purine; ACPD, (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)

-1,3-dioxolan-4-yl]purine; DG, (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine; DAPD, (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine).

Figure 3 is a graph of the effect of purine dioxolanes and AZT on colony formation of human granulocyte-macrophage precursor cells, as measured in terms of percent of cells of control versus the log of the concentration of test drug. For abreviations used, see description of Figure 2.

Figure 3 is a graph of the percent inhibition of HBV DNA replication in 2.2.15 cells on day 9 in varying concentrations of test compounds. For abreviations used, see description of Figure 2 ((-)-FTC is (-)-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane). See Table * for corresponding data.

Figure 4 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl) -1,3-dioxolan-4-yl]adenine (DAPD) in Hep2G cells. Extract was obtained at four hours after exposing cells to DAPD. 1000dmp/pmol; 80 μL injected.

Figure 5 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl) -1,3-dioxolan-4-yl]adenine (DAPD) in Hep2G cells. Extract was obtained at twelve hours after exposing cells to DAPD. 1000dmp/pmol; 145 μL injected.

Detailed Description of the invention

As used herein, the term "enantiomerically pure" refers to a nucleoside composition that includes at least approximately 95%, and preferably 97%, of a single enantiomer of that nucleoside.

The invention as disclosed herein is a method and composition for the treatment of HBV infection, in humans or other host animals, that includes administering an effective amount of one or more of the above-identified compounds, or a physiologically acceptable derivative, including a 5′ and or N^6 alkylated or acylated derivative, or a physiologically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier. The compounds of this invention either possess anti-HBV activity, or are metabolized to a compound or compounds that exhibits antiretroviral activity.

In another embodiment, the invention includes a method for the treatment of humans infected with HBV that includes administering an HBV treatment amount of a prodrug of the specifically disclosed enantiomerically pure β-D-dioxolanyl purine nucleosides. A prodrug, as used herein, refers to a pharmaceutically acceptable derivative of the specifically disclosed nucleoside, that is converted into the nucleoside on administration _in vivo_, or that has activity in itself. Nonlimiting examples are pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and the 5′ and N^6 acylated or alkylated derivatives of the active compound (alternatively referred to as "physiologically or pharmaceutically acceptable derivatives"). In one embodiment, the acyl group is a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C_1-C_{20} alkyl; alkoxyalkyl including methoxymethyl; aralkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy; a dicarboxylic acid such as succinic acid; sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl; and the mono, di and triphosphate esters.

As used herein, the term alkyl specifically includes but is not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, cyclopentyl, and cyclohexyl. As used herein, the term acyl specifically includes but is not limited to acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate,propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic. The nucleoside can also be provided as a 5′ ether lipid, as disclosed in the following references, which are incorporated by reference herein: Kucera, L.S., N. Lyer, E. Leake, A. Raben, Modest E.J., D. L.W., and C. Piantadosi. 1990. Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. AIDS Res Hum Retroviruses. **6**:491-501; Piantadosi, C., J. Marasco C.J., S.L. morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. lyer, C.A. Wallen, S. Piantadosi, and E.J. Modest. 1991-Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity. J Med Chem. **34**:1408-1414; Hostetler, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 31-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 31-deoxythymidine. Antimicrob Agents Chemother. **36**:2025-2029; Hostetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman. 1990. Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides. J. Biol Chem. **265**:6112-7.

The β-dioxolanyl purine nucleoside can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. The nucleoside or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment +with an appropriate base. The ester or salt can be converted into the parent nucleoside, for example, by hydrolysis.

The active compound can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of the nucleosides that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with cations such as sodium, potassium, zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylene-diamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

Modifications of the active compound, specifically at the N^6 and 5′-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including anti-HBV or anti-HIV agents.

I. Preparation of Enantiomerically Pure Dioxolane Nucleosides

Enantiomerically pure ß-D-dioxolane-nucleosides can be prepared as disclosed in detail below, and as described in PCT/US91/09124. The process involves the initial preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-ox-ymethyl)-dioxolane from 1,6-anhydromannose, a sugar that contains all of the necessary stereochemistry for the enantiomerically pure final product, including the correct diastereomeric configuration about the 1 position of the sugar (that becomes the 4′-position in the later formed nucleoside).

The (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane is condensed with a desired heterocyclic base in the presence of $SnCl_4$, other Lewis acid, or trimethylsilyl triflate in an organic solvent such as dichloroethane, acetonitrile, or methylene chloride, to provide the stereochemically pure dioxolane-nucleoside.

In preparing enantiomerically pure dioxolane nucleosides, care should be taken to avoid strong acidic conditions that would cleave the dioxolane ring. Reactions should be performed, if possible, in basic or neutral conditions, and when acidic conditions are necessary, the time of reaction should be minimized.

Racemic mixtures of dioxolane purine nucleosides can be prepared as described in EPA Publication No. 0 382 526. The ß-L-enantiomer can be isolated from the racemic mixture by known methods, including through the use of a chiral HPLC column.

Figure I and Example 1 set out a process for the preparation of the active compounds. The starting material, compound 1, is prepared as disclosed in PCT/US91/09124 (compound 8 in that application). 2,6-Disubstituted purine derivatives were synthesized by the condensation of acetate **1** with the silylated 6-chloro-2-fluoropurine, which gave a mixture (α/ß=1/1.3) of **2** and **3**. The initially formed N^7-isomer was again converted to the N^9-isomer during stirring overnight at room temperature. The analytical sample was obtained from the separation of α,ß-mixture to the individual isomers **2** and **3** by a preparative TLC using CH_2CL_2-acetone (19:1) as the developing solvents. However, for the purpose of preparing the final products **10-15**, the mixture of **2** and **3** was treated with NH_3 in DME (Robins, M.i.; Vznanski, B. Nucleic acid related compounds. 34. Non-aqueous Diazotization with *tert*-Butyl nitrite. Introduction of fluorine, chlorine, and bromine at C-2 of purine nucleosides. *Can. J. Chem.* **1981**, 2608) to give a mixture of **10-13**, which was separated to the individual isomers **4** (24%), **5** (18.6%;), **6** (25.89.,) and **7** (16%,). The guanine **8** and 2,6-diamino **9** derivatives were prepared by the treatment of **4** with 2-mercaptoethanol/NaOMe and ammonia in ethanol, respectively. The free nucleosides **10-15** were obtained upon treatment of the corresponding 5'-silylated nucleosides with *n*-Bu_4NF in good yields. The α-isomers **12** and **13** were prepared by the similar procedure as the ß-isomers.

Example 1 Preparation of Enantiomerically Pure ß-D-Dioxolanyl Purine Nualeosides

(2R,4R) and (2R,4S)-9-[[2-[(*tert*-Butyldiphenylsilyl) oxylmethyl]- 1,3-dioxolan-4-yl]-6-chloro-2-fluoropurine (2 and 3).

A mixture of 2-fluoro-6-chloropurine (4.05 g, 23.47 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (940 mL) was refluxed for 2 hours. The resulting solution was concentrated under anhydrous conditions to yield silylated 2-fluoro-6-chloropurine as a white solid. To a cooled (OOC) and stirred solution of silylated 2-fluoro-6-chloropurine (5.69 g, 23.69 mmol) and compound **1** (7.84 g, 19.57 mmol) in dry methylene chloride (175 mi) was added TMSOTF (4.41 mL, 23.44 mmol). The reaction mixture was warmed to room temperature and stirred for 16 hours, during which time, all the initially formed N_7 condensed product was converted to N_9-isomer. The reaction mixture was quenched with saturated $NaHCO_3$ solution (50 mi) and stirred for an additional 20 minutes at room temperature, evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (200 mi), washed with water and brine, dried (anhydrous Na_2SO_4), filtered and evaporated to give a solid residue, which was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford a mixture of ß-anomer **8** and α-anomer **9** (1.3:1; ß/α) as a white crystalline solid (6.30 g, 62.8%). The analytical sample was purified by preparative TLC using CH_2CL_2-acetone (19:1) as the 5 developing system to give **2** (R_f= 0.50) and 3 (R_f =

0.55) for NMR characterization: UV (MeOH) μ_{max} 269.0 nm.

(-)-(2R,4R)-2-Amino-9-[[2-[(*tert*-butyldiphenylsilyl)oxylmethyl]-1,3-dioxolan-4-yl]-6-chloropurine (4), (-)-(2R,4R)-9-[[2-[(*tert-Butyldiphenylsilyl)* oxy]methyl]-1,3-dioxolan-4-yl]-2-fluoroadenine(5), (+)-(2R,4S)-2-Amino-9-[[2-[(*tert-butyldiphenylsilyl)* oxylmethyl]-1,3-dioxolan-4-yl]-6-chloropurine (6) and (+)-(2R,4S)-9-[[2-[(*tert*-Butyldiphenylsilyl)oxylmethyl]-1,3-dioxolan-4-yl]-2-fluoroadenine (7).

Dry ammonia gas was bubbled into a stirred solution of **2** and **3** (6.25 g, 12.18 mmol) in DME (125 mL) overnight). The solvent was evaporated under reduced pressure and the residue was subjected to chromatographic separation of the four compounds on a silica gel column (20-30% ethyl acetate in CH_2CL_2). **4** (R_f = 0.35, 1.49 g, 24%): a white crystalline solid. UV (MeOH) λ_{max} 309.5 nm. Anal. ($C_{25}H_{28}CIN_5O_3Si$) C, H, CI, N. 5 (R_f = 0.21, 1.12 g, 18.6%): colorless needles. UV (MeOH)A λ_{max} 261.0, 268.0 (sh) nm. Anal. ($C_{25}H_{28}FN_5O_3Si$) C, H, F, N. 6 (R_f = 0.43, 1.60 g, 25.76%): a white crystalline solid. UV (MeOH) λ_{max} 261.0, 269.0 (sh) nm. Anal. ($C_{25}H_{28}FN_5O_3Si$) C, H, F, N. 7 (R_f= 0.12, 0.96 g, 16%), a 25 microcrystalline solid. UV (methanol) λ_{max} 261.0, 269.0 (sh) nm. Anal. ($C_{25}H_{28}FN_5O_3Si$) C, H, F, N.

(-)-(2R,4R)-2-Amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine (10).

A solution of **4** (0.46 g, 0.91 mmol) in THF (20 mL) was treated with 1 M *n*-Bu_4NF/THF (1.1 mL, 1.1 mmol) to give **10** (R_f = 0.50, 0.21 g, 84%) as a crystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 307.0 nm (ε8,370) (pH 7), 307.5 (ε8,590) (pH 2), 307.0 (ε8, 800) (pH 11). Anal. ($C_9H_{10}CIN_5O_3$) C, H, Cl, N.

(-)-(2R,4R)-2-Fluoro-9-[(2-hydroxymethyl)-1,3-dioxolan-4- yl]adenine (11).

A solution of **5** (0.56 g, 1.12 mmol) in THF (20 mL) was treated with 1 M *n*-Bu_4NF/THF (1.35 mL, 1.35 mmol) to furnish **22** (0.24 g, 85%) as a white crystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 260.8 nm (ε17,010), 268.5 (sh) nm (ε13,510) (pH 7), 261. 0 (ε16,390), 268.5 (sh) (ε13,300) (pH 2), 260.8 (ε16,700), 268.5 (sh) (ε13,200) (pH 11). Anal. ($C_9H_{10}FN_5O_3$) C, H, F, N.

(-)-(2R,4R)-9-[(2-Hydroxymethyl)-1,3-dioxolan-4-yl] guanine (14).

A mixture of **4** (0.29 g, 0. 57 mmol), $HSCH_2CH_2OH$ (0.51 mL) and 1.0 M NaOMe/MeOH (11.5 mL) in MeOH (20 mL) was refluxed for 3 hours. The reaction mixture was cooled and neutralized with glacial acetic acid. The solution was evaporated to dryness, and then the residue was triturated with $CHCI_3$, filtered and the filtrate was taken to dryness to give crude compound **8** (0.21 g, 75%), which without further purification was subjected to desilylation to give compound **3** (0.07 g, 61%) as a microcrystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 252.0 (ε8,730) (pH 7), 254.4 (ε12,130), 277.5 (sh) (ε8,070) (pH 2), 264.3 (ε10,800) (pH11). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

(-)-(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1, 3-dioxolan-4-yl]adenine (15).

A steel bomb was charged with compound 4 (0.28 g, 0.55 mmol), anhydrous ethanol (20 mL) saturated with NH_3, and heated at 90°C for 6 hours. After cooling, the compound 9 (0.26 g, 95%) obtained on evaporated of the solvent in vacuo, and then desilylated according to the same procedure described for preparation of **12** to give **15** (0.10 g, 75%) as white micro needles, recrystallized from MeOH: UV (H_2O) λ_{max} 279.0 nm (ε 8,040) (pH 7), 290.0 (ε 7,070) (pH 2), 278.8 (ε 7,580) (pH 11). Anal. ($C_9H_{12}N_6O_3$) C, H, N.

(-)-(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine can be prepared by reduction of compound 10 using a variety of reducing agents, including palladium on carbon and hydrogen gas or tributyltin hydride and azabisisobutyronitrile.

II. Anti-HBV Activity of Dioxolane Nucleosides

The ability of ß-D-dioxolane-nucleosides to inhibit HBV can be measured by various experimental techniques. The assay used herein to evaluate the ability of the disclosed compounds to inhibit the replication of HBV is described in detail in Korba and Gerin, Antiviral Res. 19: 55-70 (1992). For purposes of illustration only, and without limiting the invention, below is provided the results of the evaluation of toxicity and anti-HBV activity of (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine;(-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-I,3-dioxolan-4-yl]adenine; and (-)-(2R,4R)-9-1(2-hydroxymethyl)-I,-3-dioxolan-4-yl]quanine. The other compounds disclosed herein are evaluated similarly.

The antiviral evaluations were performed on two separate passages of cells, two cultures per passage (4 cultures total). All wells, in all plates, were seeded at the same density and at the same time.

Due to the inherent variations in the levels of both intracellular and extracellular HBV DNA, only depressions greater than 3.0-fold (for HBV virion DNA) or 2.5-fold (for HBV DNA replication intermediates) from the average levels for these HBV DNA forms in untreated cells are generally considered to be statistically significant [$P<0.05$] (Korba and Gerin, Antiviral Res. 19: 55-70, 1992). The levels of integrated HBV DNA in each cellular DNA preparation (which remain constant on a per cell basis in these experiments) were used to calculate the levels of intracellular HBV DNA forms, thereby eliminating technical variations inherent in the blot hybridization assays.

Typical values for extracellular HBV virion DNA in untreated cells range from 50 to 150 pg/ml culture medium (average of approximately 76 pg/ml). Intracellular HBV DNA replication intermediates in untreated cells range from 50 to 100 pg/ug cell DNA (average approximately 74 pg/ug cell DNA). In general, depressions in the levels of intracellular HBV DNA due to treatment with antiviral compounds are less pronounced, and occur more slowly, than depressions in the levels of HBV virion DNA.

For reference, the manner in which the hybridization analyses were performed for these experiments results in an equivalence of approximately 1.0 pg intracellular HBV DNA/ug cellular DNA to 2-3 genomic copies per cell and 1.0 pg of extracellular HBV DNA/ml culture medium to 3×10^5 viral particles/ml.

Toxicity analyses were performed in order to assess whether any observed antiviral effects are due to a general effect on cell viability. The method used was based on the uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems, including HSV (herpes simplex virus) and HIV.

Details of the procedure are provided in the toxicity table legends.

The test compounds were used in the form of 40 mM stock solutions in DMSO (frozen on dry ice). Daily aliquots of the test samples were made and frozen at -20°C so that each individual aliquot would be subjected to a single freeze-thaw cycle. The daily test aliquots were thawed, suspended into culture medium at room temperature and immediately added to the cell cultures. The compounds were tested at 0.0 and 1 μM for antiviral activity. The compounds were tested for toxicity at 5 concentrations up to 300 μM.

The following abbreviations are used in the Tables: ACPD, (-)-(2R,4R)-2-amino-6- chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine; DAPD, (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-I,3-dioxolan-4-yl]adenine; and Dioxolane-G, (2R,4R)-9-[(2-hydrox-ymethyl)-1,3-dioxalan-4-yl] guanine.

Example 2 Toxicity Of Compounds

The ability of the enantiomers of ACPD, DAPD, and dioxolane-G to inhibit the growth of virus in 2.2.15 cell cultures (HepG2 cells transformed with hepatitis virion) was 15 evaluated. As illustrated in Table 1, no significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for any of the test compounds at the concentrations used for the antiviral evaluations. The test compounds were not toxic to 2.2.15 cells at 100 μM. The compounds were moderately toxic at 300 μM, however, all three compounds exhibited less toxicity at this conentration than ddC.

Toxicity analyses were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule as used for the antiviral evaluations. Each compound was tested at 4 concentrations, each in triplicate cultures. Uptake of neutral red dye was used to determine the relative level of toxicity. The absorbance of internalized dye at 510 nM (A_{510}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{510} values (+/- standard deviations) in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds. The percentage of dye uptake in the 9 control cultures on plate 40 was 100 +/- 3. At 150-190 μM 2′,3′-ddC, a 2-fold reduction in dye uptake (versus the levels observed in untreated cultures) is typically observed in these assays (Korba and Gerin, Antiviral Res. 19: 55-70, 1992).

Table 1. Toxicity analysis of test compounds in 2.2.15 cells.

NEUTRAL RED DYE UPTAKE AT INDICATED DRUG CONCENTRATION (% OF CONTROL)

PLATE	COMPOUND	1000μM	300μM	100 μM	30μM
40	2',3'-ddC	5 +/- 1	44 +/- 1	97 +/- 2	101 +/- 1

NEUTRAL RED DYE UPTAKE AT INDICATED DRUG CONCENTRATION (% OF CONTROL)

PLATE	COMPOUND	1000μM	300μM	100 μM	30μM
40	ACPD	63 +/- 1	99 +/- 1	101 +/- 2	98 +/- 2
40	DAPD	49 +/- 3	88 +/- 1	99 +/- 3	99 +/- 1
40	Dioxolane-G	56 +/- 3	88 +/- 3	101 +/- 2	100 +/- 3

Example 3 Anti-Hepatitis B Virus Activity

As indicated in Table 2, within normal variations, levels of HBV virion DNA and intracellular HBV replication intermediates [HBV RI] remained constant in the untreated cells over the challenge period. The positive treatment control, 2′,3′-dideoxycytosine [2′,3′-ddC], induced significant depressions of HBV DNA replication at the concentration used. Previous studies have indicated that 9-12 μM 2′,3′-ddC, a 90% depression of HBV RI (relative to average levels in untreated cells) is typically observed in this assay system (Korba and Gerin, Antiviral Res. 19: 55-70, 1992).

All three test compounds were potent inhibitors of HBV replication, causing depression of HBV virion DNA and HBV RI to a degree comparable to, or greater than, that observed following treatment with 2′,3′-ddC.

Table 2

EFFECT OF VARIOUS NUCLEOSIDES ON HBV PRODUCTION IN 2.2.15 CELLS

Treatment		0	3	6	9	Mono	RI
None		50	70	66	59	2.8	65
		52	56	68	70	2.8	76
		83	64	74	77	2.1	70
		67	69	99	92	2.4	83
	Mean	63.00	64.75	76.75	74.50	2.53	73.50
		15.34	6.40	15.22	13.82	0.34	7.77
DDC 10 μM		66	50	20	2	0.9	6
		58	52	13	3	1.0	4
		67	51	19	2	1.4	5
		51	48	17	2	1.1	7
	Mean	60.50	50.25	17.25	2.25	1.10	5.50
		7.51	1.71	3.10	0.50	0.22	1.29
		3.97	22.39	77.52	96.98	56.44	92.52
1.0 μM (-)- -2-NH2-6-Cl-purine-dioxolane		71	50	27	6	0.8	9
		56	52	21	3	0.8	14
		57	65	20	2	1.1	10
		69	70	16	5	1.2	11
	Mean	63.25	59.26	21.00	4.00	0.98	11.00
	S.D.	7.85	9.78	4.55	1.83	0.21	2.16
	% inhibition	-0.40	8.49	72.64	94.63	61.39	85.03
1.0 μM (-)- -2-NH2-6-Cl-purine-dioxolane		66	60	49	29	2.1	36
		51	54	39	21	2.4	33
		62	79	36	20	2.2	31
		68	84	43	17	2.6	29
	Mean	61.75	69.25	41.75	21.75	2.33	32.25
	S.D.	7.59	14.50	5.62	5.12	5.12	2.99
	% inhibition	1.98	-6.95	45.60	70.81	7.92	56.12

1.0 μM (-)- -2-NH2-6-Cl- purine-dioxolane		66	59	12	0	1.2	3
		70	45	10	1	1.4	3
		74	56	15	0	0.9	1
		61	43	11	0	1.1	2
	Mean	67.75	50.75	12.00	0.25	1.15	2.25
	S.D.	5.56	7.93	2.16	0.50	0.21	0.96
	% inhibition	-7.54	21.62	84.36	99.66	54.46	96.94
1.0 μM (-)- -2-NH2-6-Cl- purine-dioxolane		52	67	28	5	2.3	14
		58	59	34	6	2.4	11
		64	59	35	9	2.6	13
		77	62	26	8	2.1	10
	Mean	62.75	61.75	30.75	7.00	2.35	12.00
	S.D.	10.69	3.77	4.43	1.83	0.21	1.83
	% inhibition	0.40	4.63	59.93	90.60	6.93	83.67
1.0 μM (-)- -2-NH2-6-Cl- purine-dioxolane		70	86	22	2	2.0	6
		50	59	24	4	1.9	6
		56	56	23	2	1.4	3
		73	62	20	3	2.1	4
	Mean	62.25	66.75	22.25	2.75	1.85	4.75
	S.D.	113.72	1.71	0.96	0.31	1.50	
	% inhibition	1.19	-1.54	71.01	96.31	26.73	93.54
1.0 μM (-)- -2-NH2-6-Cl- purine-dioxolane		51	77	60	18	2.0	28
		59	62	70	12	2.2	23
		74	73	69	14	2.8	25
		67	61	82	11	2.4	20
	Mean	62.75	68.25	70.25	13.75	2.35	24.00
	S.D.	9.95	7.97	9.03	3.10	0.34	3.37
	% inhibition	0.40	-5.41	8.47	81.54	6.93	67.35

* Analysis of intracellular HBV DNA was 24 hours following the 9th day of treatment. DNA in each cell DNA preparation were used o calculate the levels of episomal 3.2kB HBV genomes (MONO.) and HBV DNA replication intermediates [RI].

** A "zero" indicates an undetectable level of HBV DNA, sensitivity cutoff was 0.1 pg[m].

Example 3 Toxicity in Human Erythroid (BFU-E) Precursor Cells

Figure 2 is a graph of the effect of selected purine dioxolanes and AZT on colony formation of human erythroid (BFU-E) precursor cells, as measured in percent of control cells versus the concentration in μM. As indicated, the four purine dioxolanyl nucleosides tested, APD, (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1, 3-dioxolan-4-yl]purine; ACPD, (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine; DG, (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine; and DAPD, (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine), appear to be significantly less toxic than AZT in this cell line.

Example 4 Effect on Colony Formation of Human Granulocyte-Macrophage Precursor Cells

Figure 3 is a graph of the effect of ACPD, DG, DAPG, DG and AZT on colony formation of human granulocyte-macrophage precursor cells, as measured in terms of percent of cells of control versus the log of the concentration of test drug. As indicated, the purine dioxolanyl nucleosides appear to be significantly less toxic, i.e., have a higher IC_{50}, than AZT in this cell line.

Example 5 Effect on HBV DNA Replication

Figure 4 is a graph of the percent inhibition of HBV DNA replication in 2.2.15 cells on day 9 in varying concentrations of test compounds, using a narrower range of concentration than that used in Example 1. Table 3 provides the HBV virion and HBV

RI EC_{50} and EC_{90}, cytotoxicity and selectivity index for DG, DAPG, ACPD, FTC, and DDC.

Table 3

EFFECT OF D-DDC, (-)-FTC, DG, DAPD, AND ACPD AGAINST HEPATITIS B VIRUS IN TRANSFECTED HEPG-2 (2.2.15) CELLS ON DAY 9

Compound	HBV virion[a]		HBV RI [b]		Cytotoxicity	Selectivity Index	
	EC50 ± SD µM	EC90 ± SD µM	EC50 ± SD µM	EC90 ± SD µM	IC50 ± SD µM	IC50/EC90 Virion	RI
β-D-DDC	0.39 ± 0.07	8.0 ± 0.9	1.1 ± 0.11	14.0 ± 1.4	290 ± 24	36	21
(-)-β-L-FTC	0.07 ± 0.007	2.1 ± 0.2	0.27 ± 0.02	3.6 ± 0.4	1200 ± 65	571	333
(-)-β-D-DG (CS-437)	0.68 ± 0.08	2.8 ± 0.4	0.97 ± 0.11	4.5 ± 0.5	1337 ± 99	478	297
(-)-β-D-DAPD (CS-436)	0.009 ± 0.001	1.0 ± 0.2	0.09 ± 0.01	2.2 ± 0.3	2600 ± 200	2600	1180
(-)-β-D-ACPD (CS-432)	0.001 ± 0.0002	0.89 ± 0.10	0.03 ± 0.004	1.8 ± 0.2	940 ± 83	1056	522

a) Extracellular DNA
b) Replicative intermediates (Intracellular DNA)

Example 6

Figure 5 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (DAPD) in HepG2 cells. Extract was obtained at four hours after exposing cells to DAPD (1000 dmp/pmol; 80 μL injected). The data indicates that the compound is primarily metabolised intracellularly to the triphosphate form.

Example 7

Figure 6 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (DAPD) in HepG2 cells. Extract was obtained at twelve hours after exposing cells to DAPD (1000 dmp/pmol; 145 μL injected). The data indicates that after four hours of incubation with the tritiated compound, there are high intracellular levels of the triphosphate.

IV. Preparation of Pharmaceutical Compositions

The compounds disclosed herein and their pharmaceutically acceptable salts, prodrugs, and derivatives, are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

Humans suffering from any of these conditions can be treated by administering to the patient an effective amount of (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine; (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine; (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine; or (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine or a pharmaceutically acceptable derivative or salt thereof, optionally in a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount without causing serious toxic effects in the patient treated.

A preferred dose of the active compound for all of the above-mentioned conditions will be in the range from about 1 to 60 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more 5 generally 0.1 to about 100 mg per kilograin body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably

70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 µM, preferably about 1.0 to 10 µM. This may be achieved, for example, by the intravènous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient. The concentration of active compound in the drug composition will depend on absorpeion, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agentsuch as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The active compound or pharmaceutically acceptable salt or derivative thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including anti-HBV, anti-cytomegalovirus, or anti-HIV agents.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such fomulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

V. Preparation of Phosphate Derivatives of J&-D-Dioxolane-Nucleosides

Mono, di, and triphosphate derivative of E-D-dioxolane-nucleosides can be prepared as described below.

The monophosphate can be prepared according to the procedure of Imai et al., J. Org. Chem., 34(6), 1547-1550 (June 1969). For example, about 100 mg of ß-D-dioxolane-nucleoside and about 280 µl of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0°C for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium-(ß-D-dioxolane-nucleoside)-5'-monophosphate.

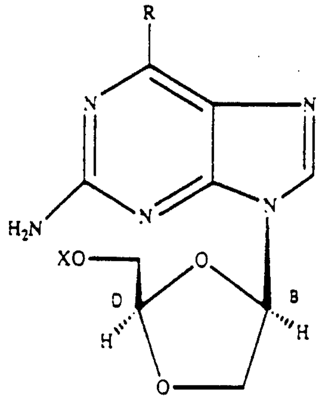
The diphosphate can be prepared according to the procedure of Davisson et al., J. Org. Chem., 52(9), 1794-1801 (1987). ß-D-Dioxolane-nucleosides can be prepared from the corresponding tosylate, that can be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

The triphosphate can be prepared according to the procedure of Hoard et al., J. Am. Chem. Soc., 87(8), 1785-1788 (1965). For example, ß-D-dioxolane-nucleoside is activated (by making a imidazolide, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, enantiomerically pure ß-D-dioxolane-nucleosides, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

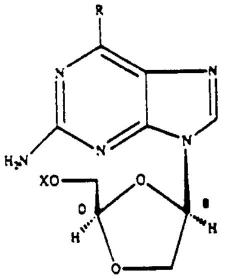
I claim.

1. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of an enantiomerically pure ß-D-dioxolanyl nucleoside of the structure:



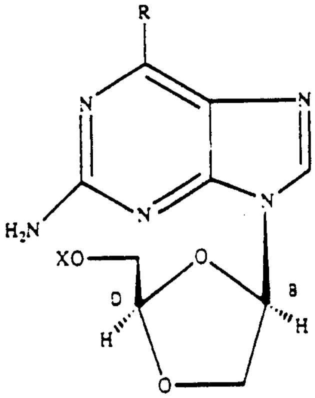
wherein R is OH, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 95% free of the corresponding ß-L enantiomer.

2. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of an enantiomerically pure ß-D-dioxolanyl nucleoside of the structure:



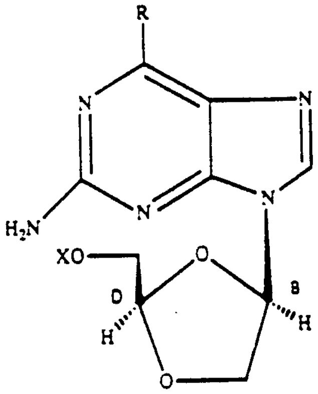
wherein R is NH_2, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 95!k free of the corresponding ß-L enantiomer.

3. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of an enantiomerically pure ß-D-dioxolanyl nucleoside of the structure:



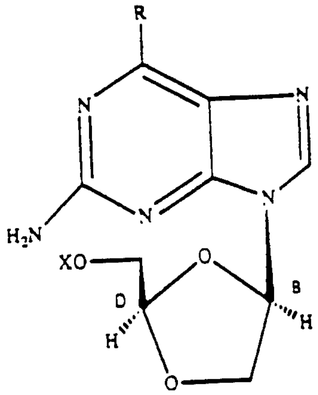
wherein R is H or Cl, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 95% free of the corresponding ß-L enantiomer.

4. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the racemic mixture of a ß-dioxolanyl nucleoside of the structure:



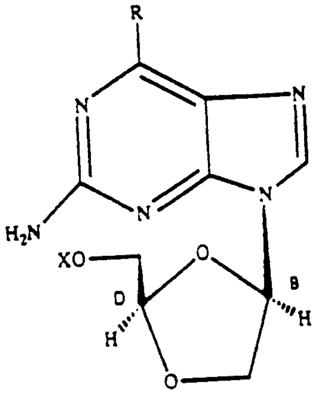
wherein R is OH, and X is selected from the group consisting ot alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt.

5. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the racemic mixture of a ß-dioxolanyl nucleoside of the structure:



wherein R is NH_2, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt.

6. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the racemic mixture of a ß-dioxolanyl nucleoside of the structure:



wherein R is H or Cl, and X is selected trom the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt.

7. The method of claims 1, 2, 3, 4, 5, or 6 wherein the carrier is suitable for oral delivery.

8. The method of claims 1, 2, 3, 4, 5, or 6 wherein the carrier comprises a capsule.

9. The method of claims 1, 2, 3, 4, 5, or 6 wherein the carrier is in the form of a tablet.

10. The method of claims 1, 2, 3, 4, 5, or 6 wherein the administration is parenteral.

11. The method of claims 1, 2, 3, 4, 5, or 6, wherein the alkyl group is selected from the group consisting of methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, cyclopentyl, and cyclohexyl.

12. The method of claims 1, 2, 3, 4, 5, or 6, wherein the acyl group is selected from the group consisting of acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic.

13. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 1 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an

enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

14. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 2 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

15. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 3 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU) ; an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

16. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 4 in alternative dosages with a compound selected from the group consisting of the (-)[]-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-

oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU) ; an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

17. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 5 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU) ; an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

18. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 6 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-I,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluorc-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

19. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 1 in combination with a compound selected from the group consisting of the

(-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydrox-ymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

20. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 2 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-flucrocytosin-1-yl)-1,3-oxathi olane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-S-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

21. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 3 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

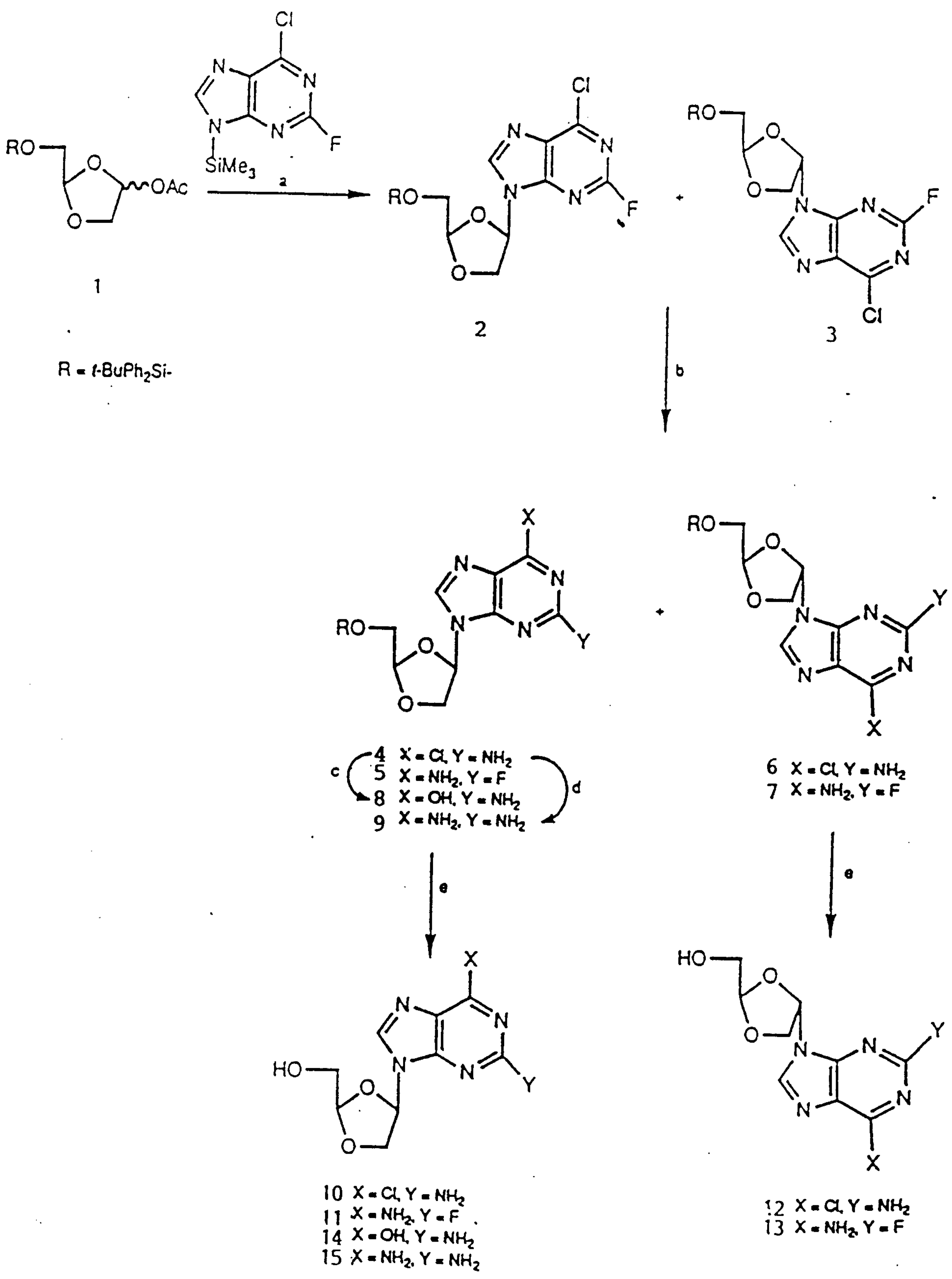
22. A method for the treatment of HIBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 4 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-I,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

23. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 5 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-I,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

24. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HAV treatment amount of the nucleoside of claim 6 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-

oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2′-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

Figure 1



[1] Reagents: (a) TMSOTf, CH_2Cl_2; (b) NH_3, DME; (c) $HSCH_2CH_2OH$, NaOMe; (d) NH_3, EtOH; (e) n-Bu_4NF, THF.

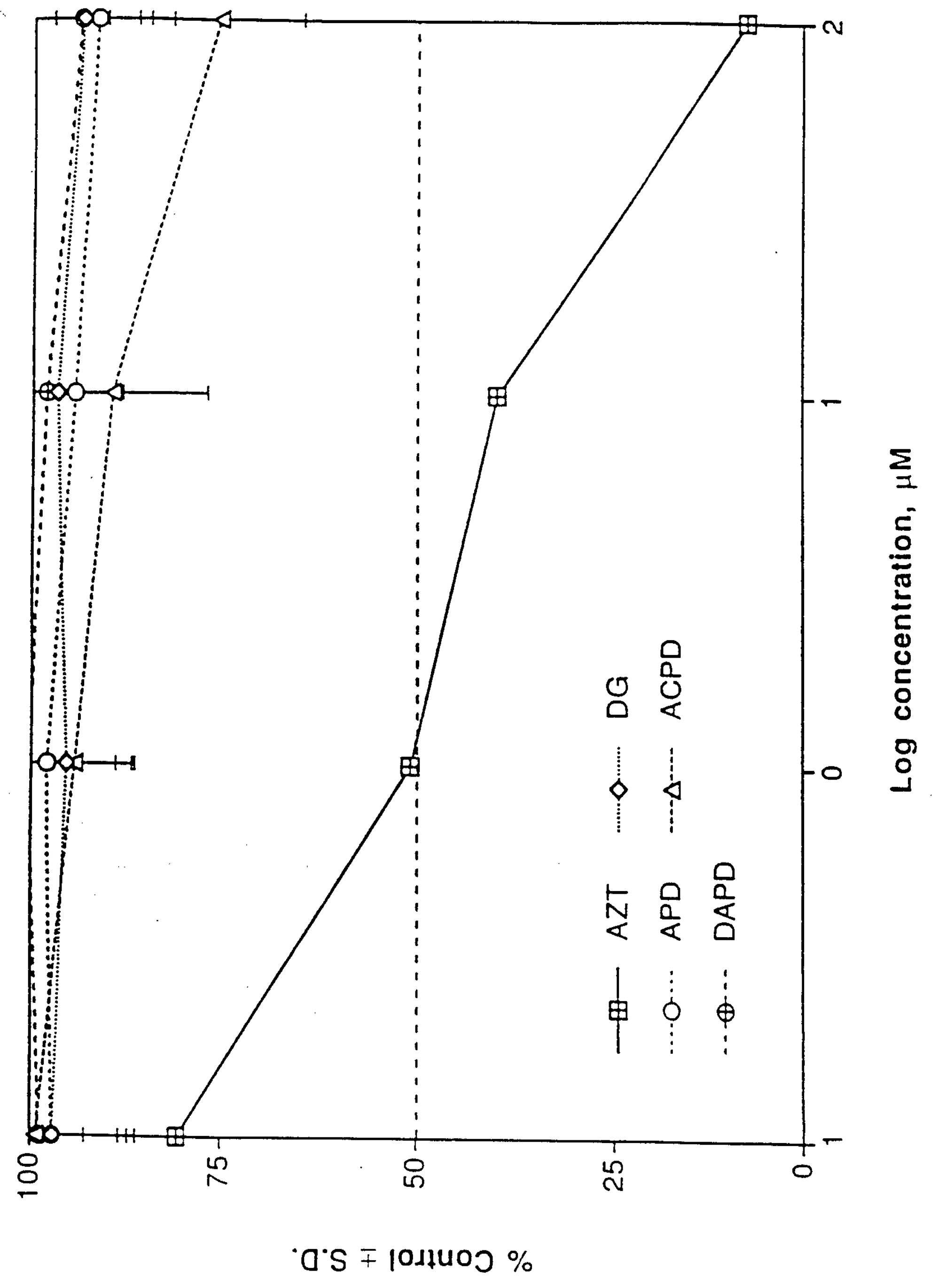

% Control ± S.D.
100
75
50
25
0
-1
0
1
2
Log concentration, µM
AZT
DG
APD
ACPD
DAPD

Figure 2

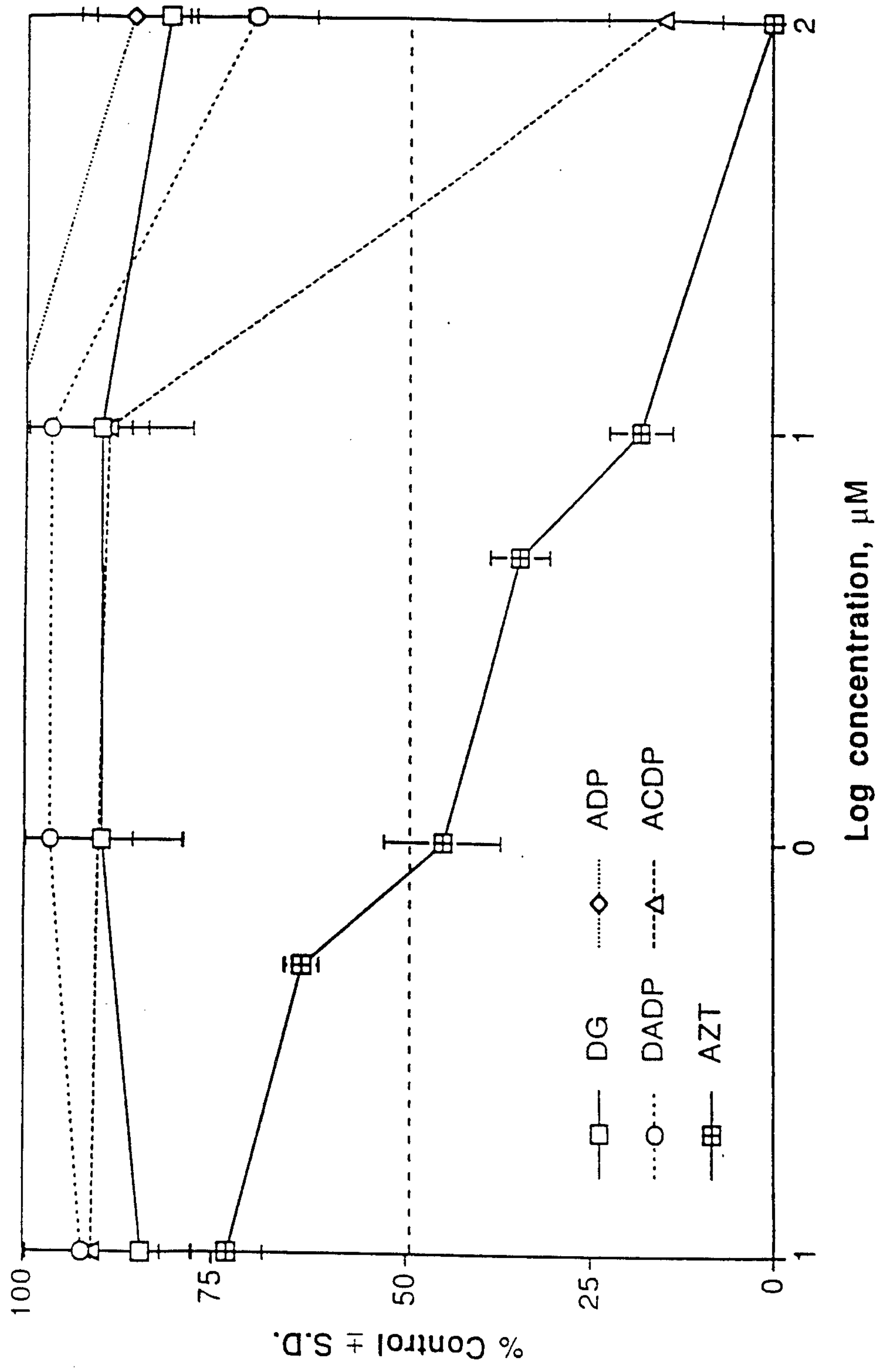

% Control ∓ S.D.
100
75
50
25
0
-1
0
1
2
Log concentration, μM
DG
ADP
DADP
ACDP
AZT

Figure 3

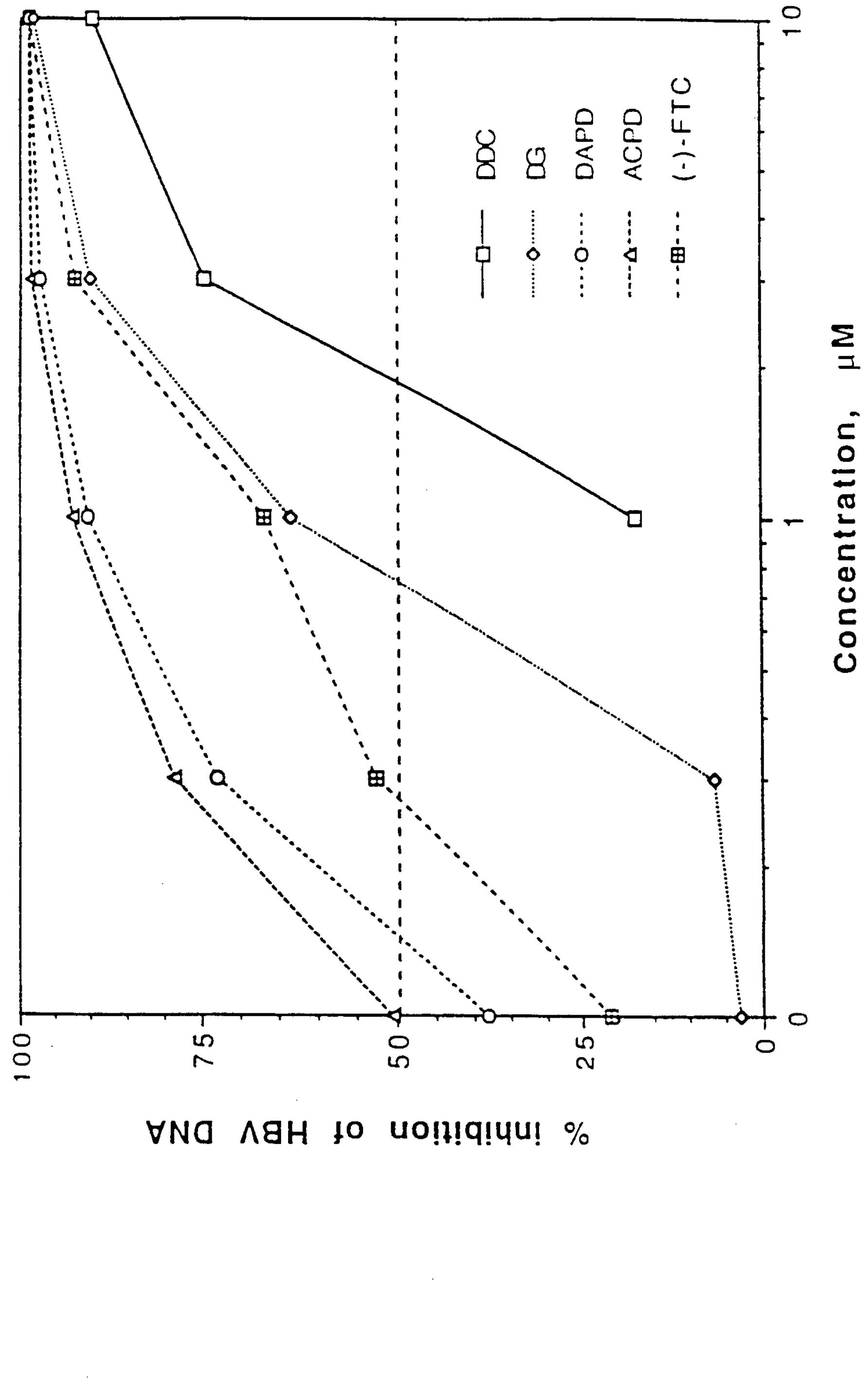

% inhibition of HBV DNA
100
75
50
25
0
0
1
10
Concentration, μM
DDC
DG
DAPD
ACPD
(-)-FTC

Figure 4

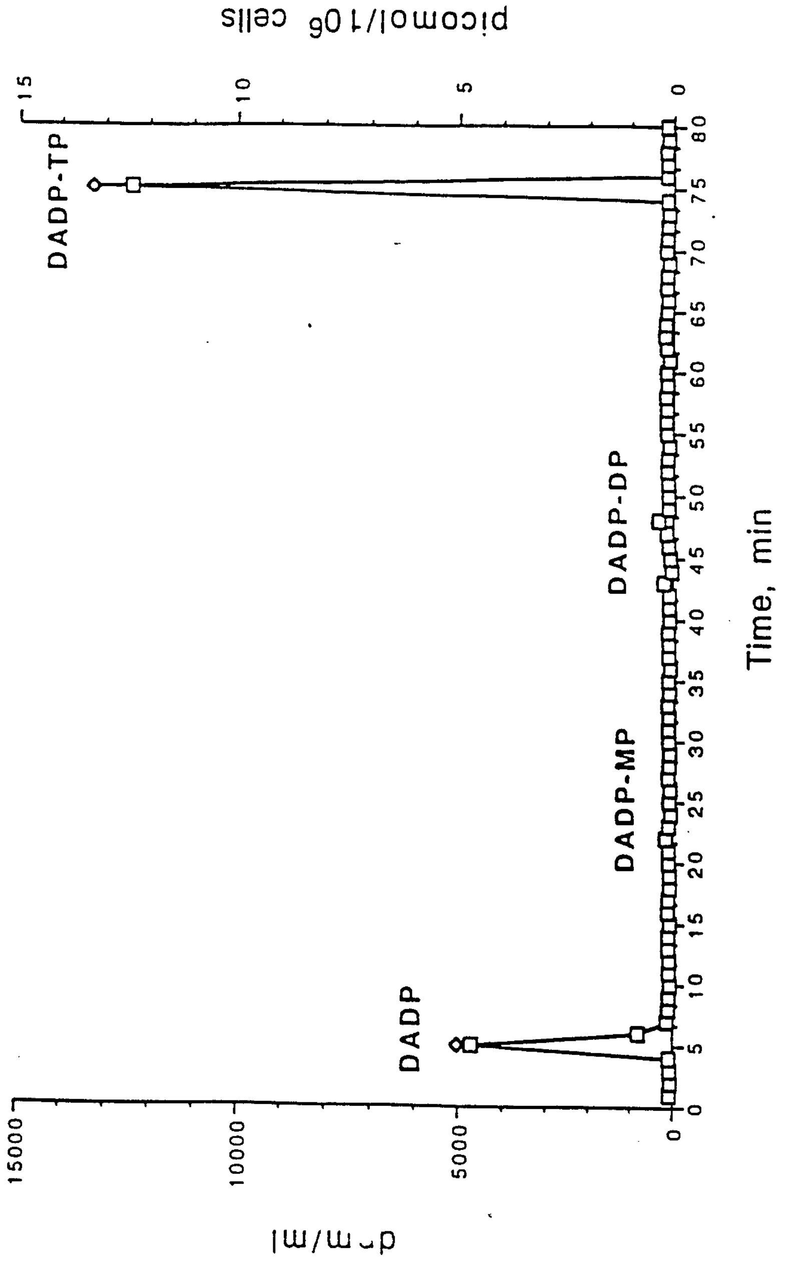

DADP
DADP-MP
DADP-DP
DADP-TP
dpm/ml
15000
10000
5000
0
picomol/10^6 cells
15
10
5
0
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time, min

Figure 5

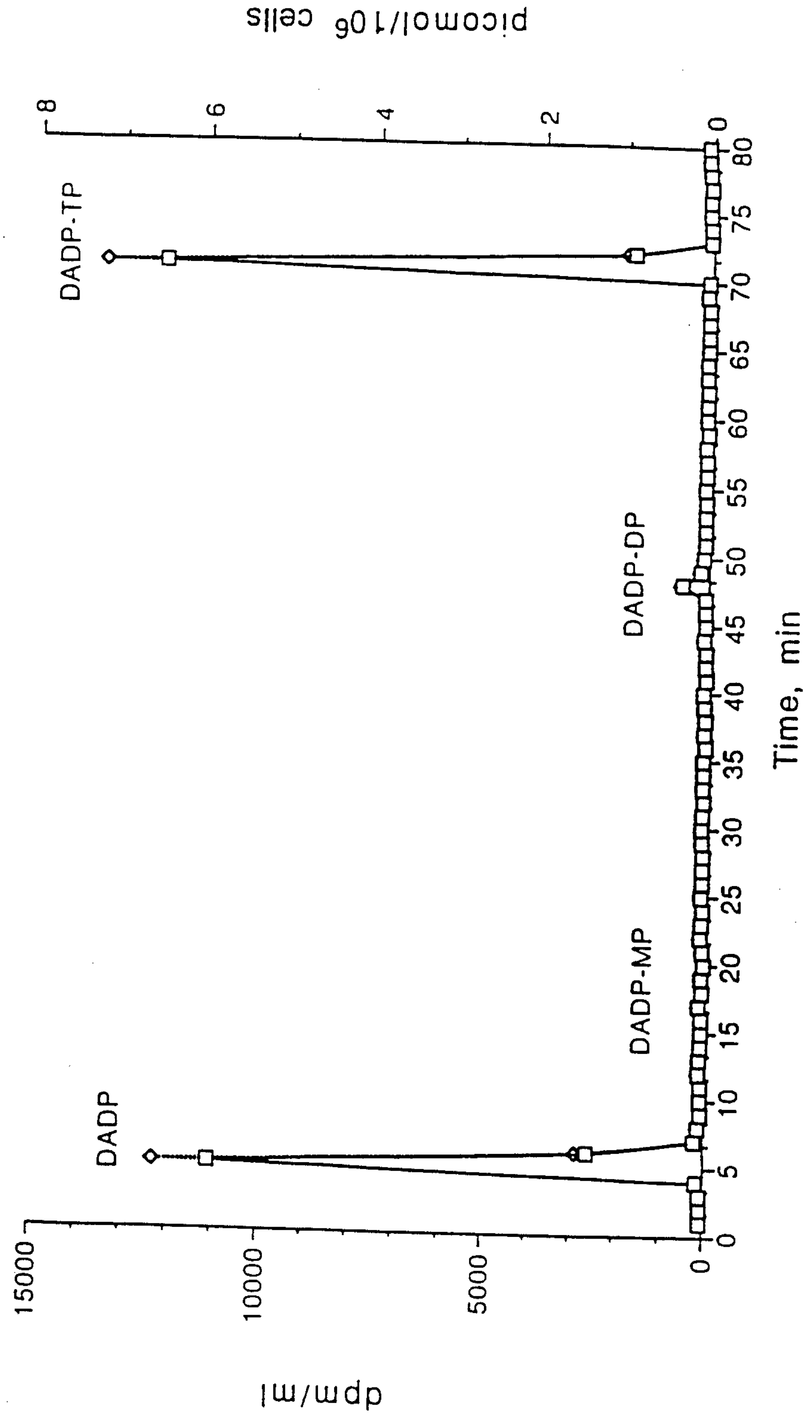

picomol/10⁶ cells
8
6
4
2
0
DADP-TP
DADP-DP
DADP-MP
DADP
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time, min
15000
10000
5000
0
dpm/ml

Figure 6

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/US 93/10348

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 337 713 (IAF BIOCHEM INTERNATIONAL INC.) 18 October 1989 see the whole document ---	1-24
Y	WO,A,92 10497 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION) 25 June 1992 cited in the application see abstract; claims ---	1-24
Y	WO,A,92 08717 (IAF BIOCHEM INTERNATIONAL INC.) 29 May 1992 see abstract see page 40, line 35 - page 41, line 24; claims 1-17; examples 18-21 --- -/--	1-24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 1994	11.02.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoff, P

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 93/10348

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTIVIRAL RESEARCH vol. 17, no. S1 , March 1992 page 44 C.K. CHU ET AL. 'SYNTHESIS AND ANTI-HIV AND ANTI-HBV ACTIVITY OF ENANTIOMERICALLY PURE OXATHIOLANE NUCLEOSIDES' see the whole document ---	1-24
P,Y	ANTIVIRAL RESEARCH vol. 20, no. S1 , April 1993 page 146 C.K. CHU ET AL. 'SYNTHESIS AND BIOLOGICAL EVALUATION OF D-(2S) AND L-(2R)-1,3-OXATHIOLANYL AND D-(2R)- AND L-(2S)-1,3-DIOXOLANYL-NUCLEOSIDES AS ANTI-HIV OR ANTI-HBV AGENTS' ---	1-24
P,Y	WO,A,92 18517 (YALE UNIVERSITY) 29 October 1992 see abstract; claims ---	1-24
A	WO,A,92 14743 (EMORY UNIVERSITY) 3 September 1992 cited in the application see abstract; claims ---	13-24
A	EP,A,0 361 831 (THE WELLCOME FOUNDATION LIMITED) 4 April 1990 see abstract see page 2, line 30 - line 53; claims ---	13-24
A	US,A,4 140 761 (GERIN ET AL.) 20 February 1979 see abstract; claims -----	13-24

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10348

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-24 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte.. onal Application No
PCT/US 93/10348

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0337713	18-10-89	AU-B- 631786	10-12-92
		AU-A- 3264489	12-10-89
		JP-A- 1316375	21-12-89
		OA-A- 9470	15-11-92
		US-A- 5041449	20-08-91
		US-A- 5270315	14-12-93
WO-A-9210497	25-06-92	US-A- 5179104	12-01-93
		AU-A- 9125991	08-07-92
		AU-A- 9147591	08-07-92
		EP-A- 0562009	29-09-93
		WO-A- 9210496	25-06-92
		US-A- 5248776	28-09-93
WO-A-9208717	29-05-92	AU-A- 8864191	11-06-92
		CA-A- 2095613	14-05-92
		EP-A- 0560794	22-09-93
WO-A-9218517	29-10-92	NONE	
WO-A-9214743	03-09-92	US-A- 5210085	11-05-93
		AU-A- 1437292	15-09-92
		AU-A- 1561792	15-09-92
		CN-A- 1065065	07-10-92
		EP-A- 0575482	29-12-93
		WO-A- 9214729	03-09-92
EP-A-0361831	04-04-90	JP-A- 2129125	17-05-90
US-A-4140761	20-02-79	NONE	