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(54) Title: METHOD OF TREATING OR PREVENTING HEPATITIS B VIRUS

(57) Abstract

Anti-hepatitis B virus compounds (-)3′-thia-2′,3′-dideoxycytidine, (-)5-fluoro-3′-thia-2′,3′-dideoxycytidine, (±) β-dioxolane cytosine and (-)-L-β-dioxolane cytosine. A method of treating a patient suffering from hepatitis B virus or preventing hepatitis B virus infection comprising administering to the patient an effective amount of an active compound selected from the group consisting of (a) (-)3′-thia-2′,3′-dideoxycytidine, (b) (±)3′-thia-2′,3′-dideoxycytidine, (c) (-)5-fluoro-3′-thia-2′,3′-dideoxycytidine, (d) (±)5-fluoro-3′-thia-2,3-dideoxycytidine, (e) (±) β-dioxolane cytosine and (f) (-)-L-β-dioxolane cytosine, or a salt or an ester thereof, either alone or in admixture within a diluent.

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METHOD OF TREATING OR PREVENTING HEPATITIS B VIRUS

GOVERNMENT RIGHTS

This invention was made with United States Government support under Grant CA-44358 from the National Cancer Institute (NIH). Accordingly, the United States Government has certain rights in this invention.

CROSS-REFERENCES TO RELATED APPLICATIONS

This is a continuation-in-part application of application Serial No. 07/785,545, filed on October 31, 1991, which in turn is a continuation-in-part application of Serial No. 07/718,806, filed June 21, 1991, which in turn is a continuation-in-part application of application Serial No. 07/686,617, filed April 17, 1991, now abandoned.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention concerns (-)3'-thia-2'3'-dideoxycytidine and (-)5-fluoro-3'-thia-2',3'-dideoxycytidine, a method for preparing the same and the use of the same or (±)3'-thia-2',3'-dideoxycytidine or (±)5-fluoro-3'-thia-2',3'-dideoxycytidine in a method for treating patients having hepatitis B virus or to prevent hepatitis B virus infection.

The present invention also relates to dioxolane-cytosine and particularly (-)-L-ß-dioxolane-cytosine and its use in a method for treating patients having hepatitis B virus or to prevent hepatitis B virus infection.

Background Information

Hepatitis B virus (HBV) causes acute or chronic hepatitis which affects nearly 300 million people worldwide (Ayoola, E.A., Balayan. M.S., Deinhardt, F. Gust, I. Kureshi, A.W., Maynard, J.E., Nayak, N.C., Bordley, D.W., Ferguson, M. Melnick, J., Purcell, R.H. and Zuckerman, A.J., Bull. World Health. Org., 66: 443-455, 1988). Chronic infection with HBV has been associated with a high risk for the development of primary hepatocellular carcinoma (Beasley, R.P., Hwang, L.Y., Lin, C.C., and Chien, C.S., "Hepatocellular Carcinoma and Hepatitis B Virus," Lancet ii: 1129-1133, 1981; Di Bisceglei, A.M., Rustgi, V.K., Hoofnagle, J.H., Dusheik, G.M., and Lotze, M.T., "Hepatocellular Carcinoma," Ann. Intern. Med. 108: 390-401, 1988).

Effective antiviral therapy against HBV infection has not been fully developed. It has been hampered by the extremely narrow host range and limited access to experimental culture systems. Lately, Hepadnaviruses have been propagated in tissue culture (Sureau, C., Romet-Lomonne, J.L., Mullins, J.I., and Essex, M., "Production of Hepatitis B Virus by a Differentiated Human Hepatoma Cell Line after Tranfection with Cloned Circular HBV DNA," Cell 47:37-47; 1986; Chang, C., Jeng, K.S., Hu, C.P., Lo, S.J., Su, T.S., Ting, L.P., Chou, C.K., Han, S.H., Pfaff, E., Salfeld, J., and Schaller, H., "Production of Hepatitis B Virus in Vitro by Transient Expression of Cloned HBV DNA in a Hepatoma Cell Line," EMBO J. 6: 675-680, 1987; Tsurimoto, T., Fujiyama, A., and Matsubara, K., "Stable Expression and Replication of Hepatitis B Virus Genome in an Integrated State in a Human Hepatoma Cell Line Transfected with the Cloned Viral DNA," Proc. Natl. Acad. Sci. USA, 84: 444-448, 1987; and Sells, M.A., Chen, M.L., and Acs, G., "Production of Hepatitis B Virus Particles in

Hep G2 Cells Transfected with Cloned Hepatitis B Virus DNA," Proc. Natl. Sci. USA, 84: 1005-1009, 1987, making it possible to study various aspects of the viral life cycle and screening for antiviral drugs.

Hepadnaviruses replicate through a ribonucleic acid (RNA) template that requires reverse transcriptase activity (Ganem, D., and Varmus, H.E., "The Molecular biology of the hepatitis B viruses," Ann. Rev. Biochem. 56: 651-693, 1987). The rationale for a chemotherapeutic treatment for hepatitis B is the inhibition of the viral DNA polymerase. HBV DNA polymerase has a common evolutionary origin with the reverse transcriptase from retroviruses (Miller, R.H., and Robinson, W.S., "Common Evolutionary Origin of Hepatitis B Virus and Retroviruses," Proc. Natl. Acad. Sci. USA, 83: 2531-2535, 1986).

Inhibitors for reverse transcriptase of oncogenic RNA viruses suppress the polymerase from HBV (Matthes, E., Langen, P., von Janta-Lipinski, M., Will, H. Schroder, H.C., Merz, H., Weiler, B.E., and Muller, W.E.G., "Potent Inhibition of Hepatitis B Virus Production in Vitro by Modified Pyrimidine Nucleosides," Antimicrobial Agents and Chemotherapy, 34: 1986-1990, 1990; Lee, B., Luo, W., Suzuk, S., Robins. M. J., and Tyrrell, D.L.J., "In Vitro and in Vivo Comparison of the Abilities of Purine and Pyrimidine 2',3'-Dideoxynucleosides to Inhibit Duck Hepadnavirus," Antimicrobial Agents and Chemotherapy, 33: 336- 339, 1989). Several 2'3'-dideoxynucleoside analogs have been used as potential antiretroviral agents. 2'3-Dideoxycytidine (ddC) has been shown to be the most potent inhibitor of HIV replication in cell culture (Mitsuya, H., Yarchoan, R., and Broder, S., "Molecular Targets for AIDS Therapy," Science, 249: 1533-1544, 1990). ddC was also shown to have potent antiviral activity against duck hepatitis B virus both in

vitro (Lee, B., Luo, W., Suzuk, S., Robins, M. J., and Tyrrell, D.L.J., "In Vitro and in Vivo Comparison of the Abilities of Purine and Pyrimidine 2',3'-dideoxynucleosides to Inhibit Duck Hepadnavirus," Antimicrobial Agents and Chemotherapy, 33: 336-339, 1989) and in vitro (Kassianides, L., Hoofnagle, J. H., Miller, R.H., Doo, E., Ford, H., Broder, S., and Mitsuya, H., "Inhibition of Duck Hepatitis B Virus Replication by 2',3'-Dideoxycytidine," Gastroenterology, 97: 1275-1280, 1989).

Studies have indicated that elimination of mitochondria DNA by treatment of ddC is related to delayed cytotoxicity and possibly resulted in peripheral neuropathy observed in clinics (Chen, C.H., Cheng, Y.C., "Delayed Cytotoxicity and Selective Loss of Mitochondria DNA in Cells Treated with the Anti-Human Immunodeficiency Virus Compound 2',3'-Dideoxycytidine," J. Biol. Chem., 264: 11934-11937, 1989).

EP 382 526, the entire contents of which are incorporated by reference herein, concerns substituted 1,3-oxathiolanes which are said to have antiviral properties. However, only retroviruses are specifically discussed and the only specific viral disorders described are human immunodeficiency virus (HIV) (AIDS), AIDS related conditions such as AIDS-related complex (ARC), persistent lymphadenopathy (PGL), AIDS-related neurological conditions (such as dementia), Kaposi's sarcoma and thrombocytopenia purpurea.

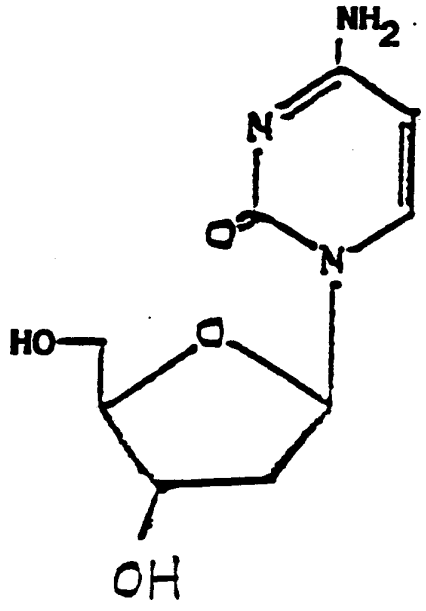
The use of BCH-189 ((±)-2',3'-dideoxy-3'-thiacytidine) and dioxolane-T as anti HIV agents are discussed in Chu et al, J. Org. Chem., 56, 6503-6505, (1991); Jeong et al, Tetrahedron Letters, 33, 595-598, (1992); Chu et al, Tetrahedron Letters, 32, 3791-3794 (1991) and B. Belleau et

al V International Conference on AIDS, Montreal, Canada, June 4-9 (1989), paper No. T.C.O.1.

BCH-189 was reported as a racemic mixture in J.A. Coates et al, Antimicrob. Agents Chemother. 36, 202 (1992) and W.-B. Choi et al, J. Am. Chem. Soc., 113, 9377 (1991).

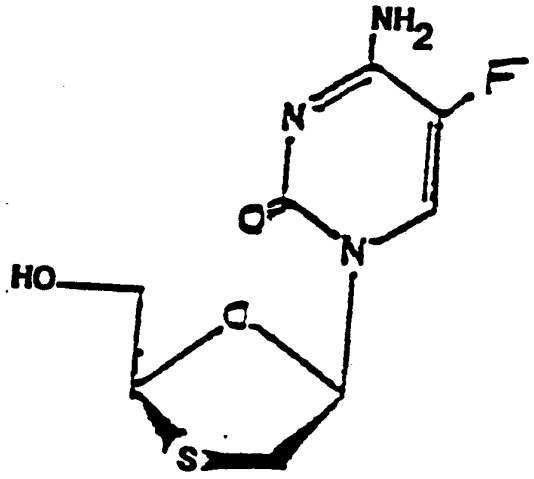
In summary, there is currently no effective treatment for human hepatitis B virus infections. Antiviral therapy and a variety of experimental drugs have had no clear benefit and some may be harmful. Recently, a variety of 2',3'-dideoxyguanosine analogues have been asserted to have anti-HBV activity in vitro, yet none have reached clinical usefulness. In general, guanosine analogues of this type may interfere with critical enzymes in humans.

NOMENCLATURE

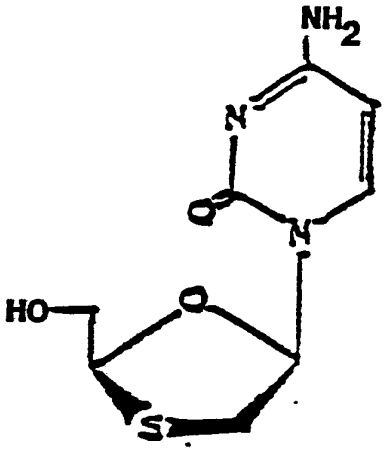


<1> dC

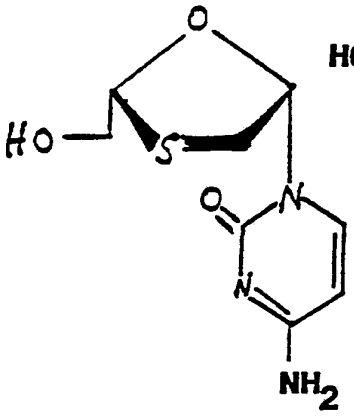
deoxycytidine



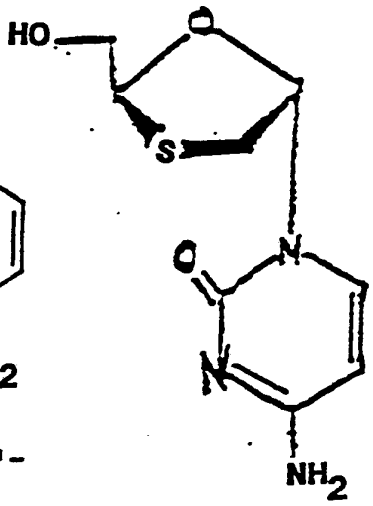
<5> + FSddc
(+)5-fluoro-3'-thia-
2',3'-dideoxycytidine



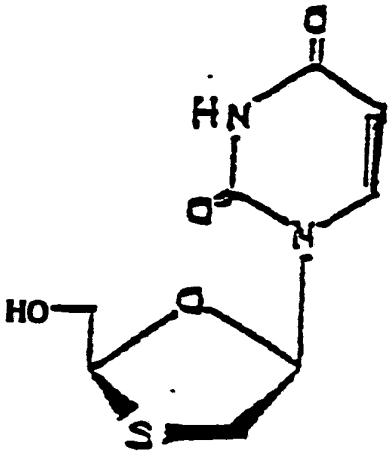
<2> + SddC
(+)3'-thia-2',3'-
dideoxycytidine



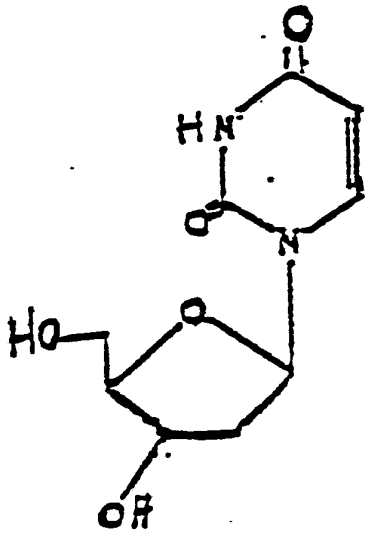
<3> -SddC
(-)3'-thia-2',3'-
dideoxycytidine



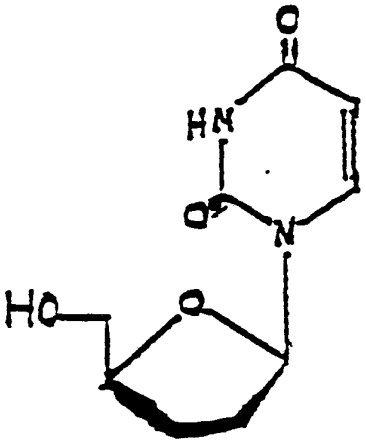
<4> α SddC
α 3'-thia-2',3'-
dideoxycytidine



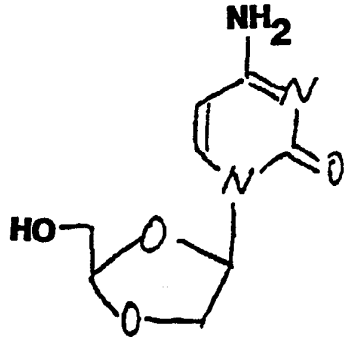
(+) SddU
(+)3'-thia-2',3'-
dideoxyuridine



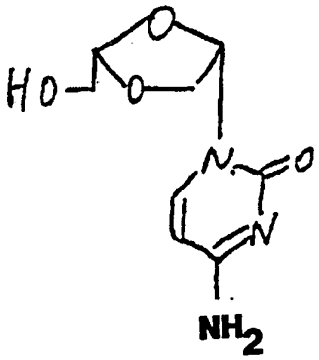
dU (2'-deoxyuridine)



ddU(2',3'-dideoxyuridine)



(+)-D-ß-dioxolane-cytosine
((+) OddC)



(-)-L-ß-dioxolane-cytosine
((-) OddC)

SddU: deaminate form of SddC (3'-thia-2',3'-dideoxycytidine)

5-FSddC: 5-fluoro-3'-thia-2'3'-dideoxycytidine

SddC: 3'-thia-2',3'-dideoxycytidine

5-MeSddC: 5-methyl-3'-thia-2',3'-dideoxycytidine

5-ClSddC: 5-chloro-3'-thia-2',3'-dideoxycytidine

5-BrSddC: 5-bromo-3'-thia-2',3'-dideoxycytidine

5-ISddC: 5-iodo-3'-thia-2',3'-dideoxycytidine

(+) OddC: (+)-D-ß-dioxolane-cytosine or (+)D-ß-dioxolane-C

(-) OddC: (-)L-ß-dioxolane-cytosine or (-)L-ß-dioxolane-C

Unless indicated to the contrary, whenever 3'-thia-2',3'-dideoxycytidine without a plus or minus sign before it is stated herein, it is understood that such means (±)3'-thia-2',3'-dideoxycytidine and whenever 5-fluoro-3'-thia-2',3'-dideoxycytidine without a plus or minus sign before it is stated herein, it is understood that such means (±)5-fluoro-3'-thia-2',3'-dideoxycytidine. Furthermore, unless indicated to the contrary, whenever ß-dioxolane-cytosine without a plus or minus sign before it is stated herein, it is understood that such means (±)ß-dioxolane-cytosine.

SUMMARY OF THE INVENTION

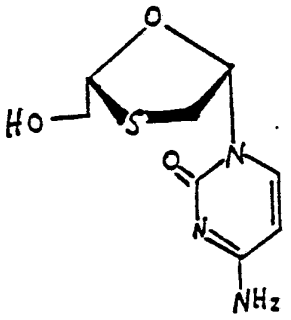
An object of the present invention is to provide (-)3'-thia-2',3'-dideoxycytidine and (-)5-fluoro-3'-thia-2',3'-dideoxycytidine and a method of preparing the same.

A further object of the present invention is to provide ß-dioxolane cytosine and particularly (-)-L-ß-dioxolane-cytosine.

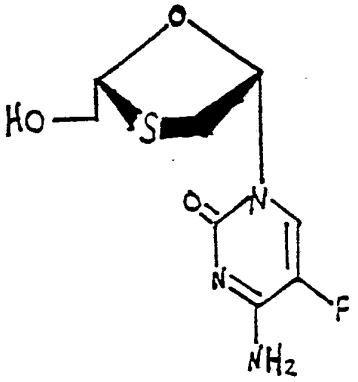
It is another object of the present invention is to treat patients suffering with the hepatitis B virus or to prevent hepatitis B virus infection in a patient.

The above stated objects, and other objects, aims and advantages are provided by the present invention.

The present invention concerns (-)3'-thia-2',3'-dideoxycytidine of the following formula:



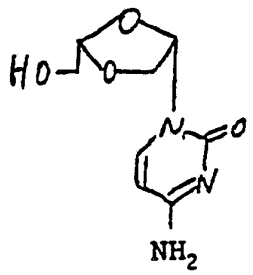
The present invention also relates to (-)5-fluoro-3'-thia-2',3'-dideoxycytidine of the following formula:



The instant invention is also directed to a method of separating (-)3'-thia-2',3'-dideoxycytidine from (±)3'-thia-2',3'-dideoxycytidine. The method comprises contacting (±)3'-thia-2',3'-dideoxycytidine with deoxycytidine deaminase, subjecting the resultant reaction mixture to column chromatography, for example, HPLC, and separating out (-)3'-thia-2',3'-dideoxycytidine. The above method is also applicable for separating (-)5-fluoro-3'-thia-2',3'-dideoxycytidine from (±)5-fluoro-3'-thia-2',3'-dideoxycytidine.

The present invention also concerns a method of treating hepatitis B virus infection or preventing hepatitis B virus infection in a patient, e.g., a mammal, e.g., a human comprising administering to the patient an effective amount of a substituted-1,3-oxathiolane compound selected from the group consisting of (-)3'-thia- 2',3'-dideoxycytidine, (±)3'-thia-2',3'-dideoxycytidine, (-)5-fluoro-3'-thia-2',3'-dideoxycytidine and (±)5-fluoro-3'-thia-2',3'-dideoxycytidine, preferably (-)3'-thia-2',3'-dideoxycytidine or (-)5-fluoro-3'-thia-2',3'-dideoxycytidine, or a salt or ester thereof, either alone or in admixture with a pharmaceutically acceptable carrier.

The present invention also concerns ß-dioxolane-cytosine and particularly (-)-L-ß-dioxolane-cytosine of the formula



(-)-L-ß-dioxolane-cytosine
((-)OddC)

The present invention also concerns a method of treating hepatitis B virus infection or preventing hepatitis B virus infection in a patient, e.g., a mammal, e.g., a human comprising administering to the patient an effective amount of a ß-dioxolane-cytosine, particularly (-)-L-ß-dioxolane cytosine, or a salt or ester thereof, either alone or in admixture with a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a Southern blot analysis of the comparative potency of deoxycytidine analogs as inhibitors of HBV 2.2.15 cells which were incubated with the various concentrations of drugs for 12 days. Media were harvested. Virions were precipitated with PEG. Nucleic acids were extracted from PEG precipitates and analyzed by Southern blot analysis. RC: Relaxed circular HBV DNAs. SS: single stranded HBV DNAs. D4C: 2',3'-didehydro-2',3'-dideoxycytidine. 3'-FddC: 3'-fluoro-2',3'-dideoxycytidine. SddC: 3'-thia-2',3'-dideoxycytidine. 5-FSddc: 5-fluoro-3'-thia-2',3'-dideoxycytidine; ddC: 2',3'dideoxycytidine.

Fig. 2 is a Southern analysis of intracellular HBV DNAs. 2.2.15 cells were untreated (lanes 1,10), treated with 5-fluoro-3'-thia-2',3'-dideoxycytidine (lanes 2,3,4,5,and 11) and 3'-thia-2',3'-dideoxycytidine (lanes 6,7,8,9,and 12) for 12 days. Total cellular DNAs were extracted as described in hereinbelow. DNAs were digested with Hind III and electrophoresed in 0.8% agarose gel, transferred to Hybond-N membrane and hybridized with ^{32}P-labeled HBV probe. Each lane represents 20 µg total cellular DNAs. Lanes 10, 11, 12: DNAs from cells untreated (lane 10) or treated with 2 µm 5-fluoro-3'-thia-2',3-dideoxycytidine (lane 11) and 3'-thia 2',3'-dideoxycytidine

(lane 12) for 12 days and further incubation in the absence of drugs for 12 more days. RC: Relaxed circular episomal HBV DNAs; I: Integrated HBV DNAs.

Fig. 3 is a Southern blot depicting the reversibility of 5-fluoro-3'-thia-2',3-dideoxycytidine and 3'-thia-2',3'-dideoxycytidine. 2.2.15 cells untreated or treated with 2 μM of 5-fluoro-3'-thia-2',3-dideoxycytidine and 3'-thia-2',3'-dideoxycytidine for 12 days were incubated with drug-free medium for 6 or 12 more days. HBV specific DNAs in the medium were analyzed as described in Fig. 1. RC: Relaxed circular HBV DNAs. SS: Single stranded HBV DNAs.

Fig. 4 is a Northern blot analysis of RNAs. Total RNAs were extracted from 2.2.15 cells untreated (lane 1) or treated with 2.0 μm 5-fluoro-3'-thia-2',3'-dideoxycytidine (lane 2) and 3'-thia-2',3'-dideoxycytidine (lane 3) for 12 days. Each lane represents 20 μg total RNAs.

Fig. 5 is a Southern blot depicting the comparative potency of various analogs of 3'-thia-2',3'-dideoxycytidine as inhibitors of HBV replication. 2.2.15 cells were treated with various analogues at 1.0 μM for 12 days. Media were analyzed for the presence of HBV DNAs as described in Fig.1.

Fig. 6A depicts a HPLC profile of a mixture of (±)3'-thia-2',3'-dideoxycytidine before a deamination.

Fig. 6B depicts a HPLC profile 16 hours after deamination of the mixture.

Fig. 7A depicts a HPLC profile of a control having only (+)3'-thia-2',3'-dideoxycytidine before deamination.

Fig. 7B depicts a HPLC profile of a UV spectrum 16 hours after deamination of (+)3'-thia-2',3'-dideoxycytidine.

Fig. 8A depicts a HPLC profile of (-)3'-thia-2',3'-dideoxycytidine before a deamination.

Fig. 8B depicts a HPLC profile of (-)3'-thia-2',3'-dideoxycytidine 16 hours after a deamination.

Fig. 9A depicts a HPLC profile of α-SddC before a deamination.

Fig. 9B depicts a HPLC profile of α-SddC 16 hours after a deamination.

Fig. 10 is a UV spectrum for 3'-thia-2',3'-dideoxycytidine.

Fig. 11 is a UV spectrum for 3'-thia-2',3'-dideoxyuridine.

Figs. 12A and Fig. 12B each depict a Southern analysis of intracellular HBV DNA wherein 2.215 cells are untreated (control) or treated.

Fig. 13 is a Southern blot analysis of the comparative potency of several compounds as inhibitors of intracellular HBV DNA after a one week incubation with HBV 2.2.15 cells.

DETAILED DESCRIPTION OF THE INVENTION

The rationale for a chemotherapeutic treatment for hepatitis B virus is the inhibition of the viral DNA polymerase. Many nucleoside analogs have been tested both in a tissue culture system and an animal model with varying success (Matthes, E., Langen, P., von Janta-Lipinski, M., Will, H. Schroder, H.C., Merz, H., Weiler, B.E., and Muller, W.E.G., "Potent Inhibition of Hepatitis B Virus Production in Vitro by Modified Pyrimidine Nucleosides," Antimicrobial Agents and Chemotherapy, 34: 1986-1990, 1990; Lee, B., Luo, W., Suzuk, S., Robins, M. J., and Tyrrell, D.L.J., "In Vitro and in Vivo Comparison of the Abilities of Purine and Pyrimidine 2',3'-Dideoxynucleosides to Inhibit Duck Hepadnavirus," Antimicrobial Agents and Chemotherapy, 33: 336-339, 1989; Kassianides, L., Hoofnagle, J. H., Miller, R.H., Doo, E., Ford, H., Broder, S., and Mitsuya, H., "Inhibition of Duck Hepatitis B Virus Replication by 2',3'-Dideoxycytidine," Gastroenterology, 97: 1275-1280, 1989; Price, P.M., Banerjee, R., and Acs, G., "Inhibition of the Replication of Hepatitis B Virus by the Carbocyclic Analogue of 2'-Deoxyguanosine," Proc. Natl. Acad. Sci., USA 86: 8541- 8544, 1989; Yokota, T., Konno, K., Chonan, E., Mochizuki, S., Kojima, K., Shigeta, S., and de Clercq, E., "Comparative Activities of Several Nucleoside Analogues Against Duck Hepatitis B Virus in Vitro," Antimicrobial Agents and Chemotherapy, 34: 1326-1330, 1990; and Ueda, K., Tsurimoto, T., Nagahata, T., Chisaka, O., and Matsubara, K., "An in Vitro System for Screening Antihepatitis B Drugs," Virology 169: 213-216, 1989).

Applicants discovered that 3'-thia-2',3'-dideoxycytidine and its 5-fluoro analog were potent compounds against HBV replication. It was further discovered that with respect to the anti-HBV effects of

(±)3'-thia-2',3'-dideoxycytidine and its racemic forms, namely (±)3'-thia-2',3'-dideoxycytidine and (-)3'-thia-2',3'-dideoxycytidine, that (-)3'-thia-2',3'-dideoxycytidine is the primary active form responsible for the anti-HBV effect and the (+)3'-thia-2',3'-dideoxycytidine is the major component causing the cytotoxicity at the concentration wherein the anti-HBV effect was observed.

In contrast to the effectiveness in inhibiting HBV replication, 3'-thia-2',3'-dideoxycytidine and 5-fluoro-3'-thia-2',3-dideoxycytidine were not found to affect the integrated HBV DNAs. Since the RNA replicative intermediates are being transcribed from the integrated DNA, it is not surprising that HBV specific transcripts were not affected by drug treatment. Thus, interruption of drug treatment resulted in a return of HBV virus to both intra- and extracellular populations.

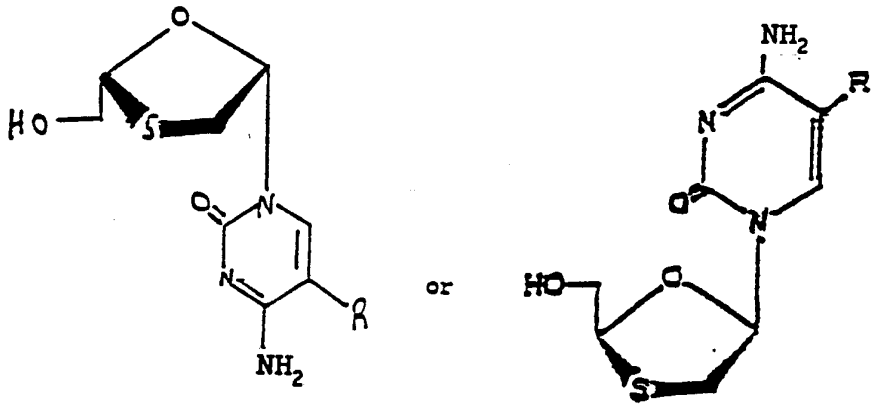
Without wishing to be bound by any particular operability, the mechanism of action of 3'-thia-2',3'-dideoxycytidine is probably (1) inhibition of viral DNA polymerase and/or (2) chain termination due to incorporation into elongated DNA strand.

3'-Thia-2',3'-dideoxyuridine analogs were found not to be active against HBV replication. There was concern that cytidine analogs can be deaminated intracellularly to inactive uracil analog. The facts that 3'-thia-2',3'-dideoxycytidine is very potent against HBV replication in 2.2.15 cells and that the anti-HBV activity is not enhanced by cyd/dcyd deaminase inhibitor suggests that catabolic inactivation by deaminase may not be important.

The present invention concerns a method involving the administration of one or more of (-)3'-thia-2',3'-

dideoxycytidine, (±)3'-thia-2',3'-dideoxycytidine, (-)5-fluoro-3'-thia-2',3-dideoxycytidine or (±)5-fluoro-3'-thia-2',3'-dideoxycytidine (referred to herein as "the compounds of formula (I)") or a salt or ester thereof, alone or in admixture with a pharmaceutically acceptable carrier in order to treat patients suffering from hepatitis B virus or to prevent hepatitis B virus infection.

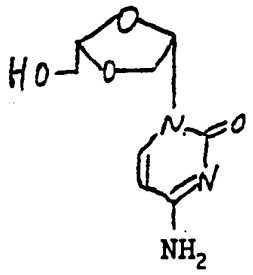
Formula (I) includes the following:



wherein R is selected from the group consisting of H and F.

As referred to herein, formula (I) refers to (-)3'-thia-2',3'-dideoxycytidine, (±)3'-thia-2',3'-dideoxycytidine, (-)5-fluoro-3'-thia-2',3'-dideoxycytidine or (±)5-fluoro-3'-thia-2',3'-dideoxycytidine or combinations thereof.

The present invention also concerns a method involving the administration of ß-dioxolane-cytosine and particularly (-)-L-ß-dioxolane-cytosine of the formula

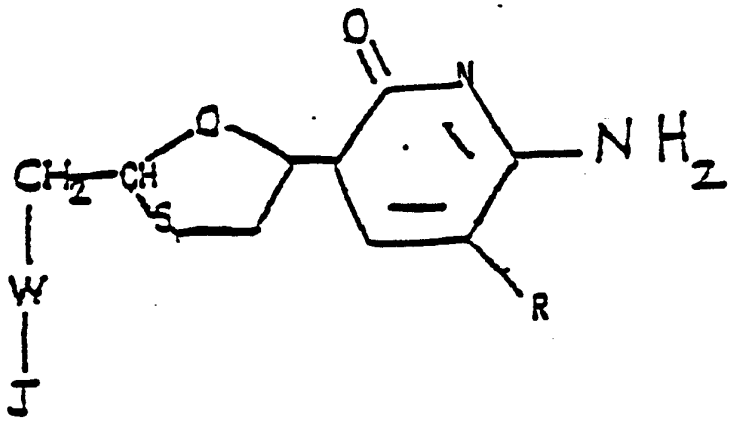


or a salt or ester thereof, alone or in admixture with a pharmaceutically acceptable carrier in order to treat patients suffering from hepatitis B virus or to prevent hepatitis B virus infection. ß-dioxolane-cytosine or (-)-L-ß-dioxolane-cytosine are hereinafter referred to as the compounds of formula (I').

Preferred esters of the compounds for use in the invention of formula (I) include the compounds in which H of $HOCH_2$ is replaced by R - $\overset{O}{\overset{\|}{C}}$ in which the non-carbonyl moiety R of the ester grouping is selected from hydrogen, straight or branched chain alkyl (e.g., methyl, ethyl, n-propyl, t-butyl, n-butyl), alkoxyalkyl (e.g., methoxymethyl), aralkyl (e.g., benzyl), aryloxyalkyl (e.g., phenoxymethyl), aryl (e.g., phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy); substituted dihydro pyridinyl (e.g., N-methyldihydro pyridinyl); sulphonate esters such as alkyl or arakylsulphonyl (e.g., methanesulphonyl); sulphate esters; amino acid esters (e.g., L-valyl or L-isoleucyl) and mono-, di- or tri-phosphate esters.

Also included within the scope of such esters are esters derived from polyfunctional acids such as carboxylic acids containing more than one carboxyl group, for example, dicarboxylic acids $HO_2C(CH_2)_nCO_2H$ where n is an integer of 1 to 10 (for example, succinic acid) or phosphoric acids. Methods for preparing such esters are well known. See, for example, Hahn et al., "Nucleotide Dimers as Anti Human Immunodeficiency Virus Agents," Nucleotide Analogues, pp.156-159 (1989) and Busso et al., "Nucleotide Dimers Suppress HIV Expression in Vitro," AIDS Research and Human Retroviruses, 4(6), pp.449-455 (1988). Where esters are derived from such acids, each acidic group is preferable

esterified by a compound for use in the invention or other nucleosides or analogues and derivatives thereof to provide esters of the formula (II)



(II)

where R is H or F, W is $-\overset{O}{\overset{\|}{O}}-C-(CH_2)_n-\overset{O}{\overset{\|}{C}}-O-$ and n is an integer of 1 to 10 or $-O-\overset{O}{\overset{\|}{P}}(O^-)-O-$ or $-O-\overset{S}{\overset{\|}{P}}(O^-)-O-$, J is any nucleoside or nucleoside analog or derivative thereof. Preferred nucleosides and nucleoside analogues are 3'-azido-2'-3'-dideoxythymidine, 2',3'-dideoxycytidine, 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine, 2',3'-dideoxythymidine, 2',3'-dideoxy-2',3'-didehydro-thymidine, and 2',3'-dideoxy-2',3'-didehydroxytidine and ribavirin.

With regard to the above described esters, unless otherwise specified, any alkyl-moiety present advantageously contains 1 to 16 carbon atoms, preferably 1 to 4 carbon atoms and could contain one or more double bonds. Any aryl moiety present in such esters advantageously comprises a phenyl group.

In particular, the esters may be a C_{1-16} alkyl ester, an unsubstituted benzoyl ester or a benzoyl ester substituted by at least one halogen (bromine, chlorine, fluorine or iodine), saturated or unsaturated C_{1-6} alkyl, saturated or unsaturated C_{1-6} alkoxy, nitro or trifluoromethyl groups.

Pharmaceutically acceptable salts of the compounds of formula (I) or formula (I') include those derived from pharmaceutically acceptable inorganic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compounds of formula (I) or formula (I') and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and NR_4+ (where R is C_{1-4} alkyl) salts.

The amount of the compound of formula (I) or formula (I') for use in the present invention will vary not only with the particular compound selected, but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately determined by the discretion of the attendant physician or veterinarian. In general, however a suitable dose will be in the range from about 1 to about 100 mg/kg of body weight per day, such as 2 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 2 to 10 mg/kg/day.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, at two, three, four or more sub-doses per day.

The compound of formula (I) or formula (I') is conveniently administered in unit dosage; for example, containing 0.5 to 50 mg, conveniently 20 to 1000 mg most conveniently 50 to 700 mg, of active ingredient (compound of formula (I) or formula (I')) per unit dosage form.

Ideally, the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 1 to 75 μM, preferably about 2 to 50 μM, most preferably about 3 to about 30 μM. This may be achieved, for example, by the intravenous injection of 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus containing about 0.1 to 50 mg/kg of the active ingredient.

While it is possible that, for use in therapy, the compound of formula (I) or formula (I') may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides for the use of a pharmaceutical formulation comprising a compound of formula (I) or formula (I') or a pharmaceutically acceptable derivative thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient therefor.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution; as a suspension; or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils) or preservatives.

The compounds of formula (I) or formula (I') may also be formulated for parental administration (e.g., by injection, for example, bolus injection or continuous

infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

For topical administration to the epidermis, the compounds according to formula (I) or formula (I') may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, suspending agents, thickening agents, or coloring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising an active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration, wherein the carrier is a solid, are most preferably represented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened

or melted carrier(s) followed by chilling and shaping in molds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient, such carriers as are known in the art to be appropriate.

Compounds of formula (I) or formula (I') or formulations containing the same can also be applied on condoms (on the inner surface thereof, outer surface thereof or both of said surfaces) to prevent the transmission of HBV during intercourse.

For intra-nasal administration, the compounds of formula (I) may be used as a liquid spray or dispersible powder or in the form of drops.

Drops may be formulated with an aqueous or non-aqueous base comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs.

For administration by inhalation, the compounds of formula (I) or formula (I') are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds of formula (I) or formula (I') may take the form of a dry powder composition, for example, a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

When desired, the above described formulations adapted to give sustained release of the active ingredient may be employed.

The pharmaceutical compositions for use according to the invention may also contain other active ingredients such as antimicrobial agents or preservatives.

The compounds of formula (I) of formula (I') may also be used in combination with other therapeutic agents, for example, other anti-infective agents. In particular, the compounds of formula (I) or formula (I') may be employed together with well known antiviral agents, e.g., adenine arabinoside or interferon α.

The invention thus provides, in a further aspect, a combination comprising a compound of formula (I) or formula (I') or a physiologically acceptable derivative thereof together with another therapeutically active agent, in particular, an anti-HBV agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus the use of pharmaceutical formulations comprising a combination as defined above together with a

pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

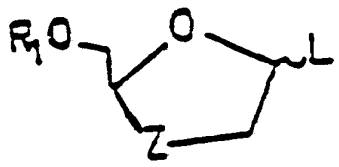
The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compound of the formula (I) or formula (I') or a pharmaceutically acceptable derivative thereof is used in combination with a second therapeutic agent active against the same virus, the dose of each compound may be either the same or different from that when the compound of formula (I) or formula (I') is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compounds of formula (I) or formula (I') and their pharmaceutically acceptable derivations may be prepared by any method known in the art for the preparation of compounds of analogous structure.

Four processes ((A) to (D)) for producing compounds of formula (I) are set forth as follows:

In one such process (A) a 1,3-oxathiolane of formula (III)



(III)

wherein R_1 is hydrogen or a hydroxyl protecting group as defined herein and the anomeric group L is a displaceable atom or group and is reacted with an appropriate base. Suitable groups L include alkoxy carbonyl groups such as ethoxy carbonyl or halogens, for example, iodine, bromine or

chlorine or -OR' where R' is a substituted or unsubstituted, saturated or unsaturated alkyl group, e.g., a C_{1-4}-alkyl group such as methyl, or R' is a substituted or unsubstituted aliphatic or aromatic acyl group, e.g., a C_{1-6}-aliphatic acyl group such as acetyl and an aromatic acyl group such as benzoyl.

The compound of formula (III) is conveniently reacted with the appropriate purine or pyrimidine base R_2-H (previously silylated with a silyating agent such as hexamethyldisilazine) in a compatible solvent such as methylene chloride using a Lewis acid (such as titanium tetrachloride or stannic chloride) or trimethyl-silytriflate.

The 1,3-oxathiolanes of formula (III) may be prepared, for example, by reaction of an aldehyde of formula (IV) with a mercaptoacetal of formula (V) in a compatible organic solvent, such as toluene, in the presence of an acid catalyst as a para-toluene sulfonic acid or a Lewis acid, e.g., zinc chloride.

$HSCH_2CH(OC_2H_5)_2$ (IV)

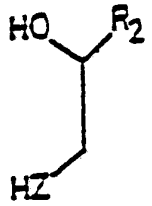
$C_6H_5COOCH_2CHO$ (V)

The mercaptoacetals of formula (IV) may be prepared by methods known in the art, for example, G. Hesse and I. Jorder, "Mercaptoacetaldehyde and dioxy-1,4-dithiane," Chem. Ber. 85 pp. 924-932 (1952).

The aldehydes of formula (V) may be prepared by methods known in the art, for example, E.G. Halloquist and H. Hibbert, "Studies on Reactions Relating to Carbohydrates and Polysaccharides, Part XLIV: Synthesis of Isomeric Bicyclic Acetal Ethers," Can. J. Research 8, pp. 129-136 (1933).

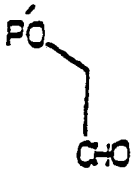
In a second process (B) one compound of formula (I) is converted to another compound of formula (I) by base interconversion. Such interconversion may be effected either by simple chemical transformation (e.g., the conversion of uracil base to cytosine) or by an enzymatic conversion using, for example, a deoxyribosyl transferase. Such methods and conditions for base interconversions are well known in the art of nucleoside chemistry.

In a third process (C) the compounds of formula (I) may be prepared by the reaction of a compound of formula (VI)



(VI)

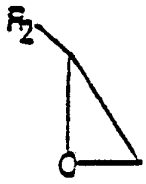
with a compound of formula (VII)



(VII)

where P is a protecting group, followed by removal of the protecting group.

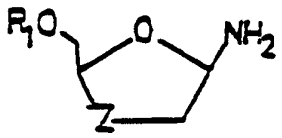
The compounds of formula (VI) may be prepared for reaction by a suitable epoxide (IX)



(IX)

with an appropriate sulphur-containing compound, e.g., sodium thioacetate. Compounds of formula (IX) are either known in the art or may be obtained by analogous processes.

In a fourth process (D) a compound of formula (X)



(X)

may be converted to a compound of formula (I) by conversion of the anomeric NH_2 group to the required base by methods well known in the art of nucleoside chemistry.

Many of the reactions described hereinabove have been extensively reported in the context of purine nucleoside synthesis, for example, in "Nucleoside Analogues - Chemistry, Biology and Medical Applications," R.T. Walker et al., Eds. Plenum Press, New York (1979) at pages 193-223, the text of which is incorporated by reference herein.

It will be appreciated that the above reactions may require the use of, or conveniently may be applied to, starting materials having protected functional groups, and deprotection might thus be required as an intermediate or final step to yield the desired compound. Protection and deprotection of functional groups may be effected using conventional means. Thus, for example, amino groups may be protected by a group selected from arakyl (e.g., benzyl), acyl or aryl (e.g., 2,4-dinitrophenyl); subsequent removal of the protecting group being effected when desired by hydrolysis or hydrogenolsis as appropriate using standard conditions. Hydroxyl groups may be protected using any conventional hydroxyl protecting group, for example, as described in "Protective Groups in Organic Chemistry," Ed.

J.F.W. McOmie (Plenum Press, 1973) or "Protective Groups in Organic Synthesis" by Theodora W. Greene (John Wiley and Sons, 1981). Examples of suitable hydroxyl protecting groups include groups selected from alkyl (e.g., methyl, t-butyl or methoxymethyl), aralkyl (e.g., benzyl, diphenylmethyl or triphenylmethyl), heterocyclic groups such as tetrahydropyranyl, acyl, (e.g., acetyl or penzoyl) and silyl groups such as trialkylsilyl (e.g., t-butyldimethylsilyl). The hydroxyl protecting groups may be removed by conventional techniques. Thus, for example, alkyl, silyl, acyl and heterocyclic groups may be removed by solvolysis, e.g., by hydrolysis under acidic or basic conditions. Aralkyl groups such as benzyl may be cleaved, for example, by treatment with BF_3 etherate and acetic anhydride followed by removal of acetate groups so formed at an appropriate stage in the synthesis. Silyl groups may also conveniently be removed using a source of fluoride ions such as tetra-n-butylammonium fluoride.

In the above processes, the compounds of formula (I) are generally obtained as a mixture of the cis and trans isomers.

These isomers may be separated, for example, by acetylation, e.g., with acetic anhydride followed by separation by physical means, e.g., chromatography on silica gel and deacetylation, e.g., with methanolic ammonia or by fractional crystallization.

With respect to the synthesis of the compounds of formula (I'), reference is made to Scheme 2 hereinafter which describes a synthesis for producing L-isomers of a dioxolane nucleoside analog.

1,6-Anhydro-L-gulose was prepared in one step from L-gulose by the treatment of L-gulose with an acid, e.g., 0.5N HCl in 60% yield (Evans, M.E., Earish, F. W., Carbohydr. Res. (1973), 28, 359). Without selective protection as was done before (Jeong, L. S.; Alves, A. J.; Carrigan, S. W.; Kim, H. O.; Beach, J. W.; Chu, C. K. Tetrahedron Lett. (1992), 33, 595 and Beach, J. W.; Jeong, L. S.; Alves, A. J.; Pohl, D; Kim, H. o.; Chang, C.-N.; Doong, S.-Li.; Schinazi, R. F.; Cheng, Y.-C.; Chu, C. K. J. Org. Chem. 1992, in press) **(2)** was directly converted to dioxolane triol **(3)** by oxidation with $NaIO_4$, followed by reduction with $NaBH_4$, which without isolation, was converted to isopropylidene derivative **(4)**. Benzoylation to **(5)**, deprotection to **(6)**, and oxidation of diol **(6)** gave the acid **(7)**. Oxidative decarboxylation of **(7)** with $Pb(OAc)_4$ in dry THF gave the acetate **(8)**, the key intermediate in good yield. The acetate was condensed with the desired pyrimidines (e.g., silylated thymine and N-acetylcytosine) in the presence of TMSOTf to afford an α,ß-mixture, which was separated on a silica gel column to obtain the individual isomers **(9-12)**. Debenzoylation with methanolic ammonia gave the desired cytosine derivative **14** and the thymine derivative **13**.

All key intermediates in Scheme 2 gave correct elemental analyses (± 0.4%).

In summary, the asymmetric synthesis of (-)-L-ß-dioxolane-C was accomplished via 8 steps from a chiral template 2.

Pharmaceutically acceptable salts of the compounds of the invention may be prepared as described in United States Patent No. 4,383,114, the disclosure of which is incorporated by reference herein. Thus, for example, when

it is desired to prepare an acid addition salt of a compound of the formula (I), the product of any of the above procedures may be converted in a salt by treatment of the resulting free base with a suitable acid using conventional methods. Pharmaceutically acceptable acid addition salts may be prepared by reacting the free base with an appropriate acid optionally in the presence of a suitable solvent such as an ester (e.g., ethyl acetate) or an alcohol (e.g., methanol, ethanol or isopropanol). Inorganic basis salts may be prepared by reacting the free base with a suitable base such as an alkoxide (e.g., sodium methoxide) optionally in the presence of a solvent such as an alcohol (e.g., methanol). Pharmaceutically acceptable salts may also be prepared from other salts, including other pharmaceutically acceptable salts of the compounds of the formula (I) using conventional methods.

A compound of formula (I) may be converted into a pharmaceutically acceptable phosphate or other ester by reaction with a phosphorylating agent, such as $POCl_3$ or a suitable esterifying agent, such as an acid halide or anhydride, as appropriate. An ester or salt of a compound of formula (I) may be converted to the parent compound, for example, by hydrolysis.

Where the compound of formula (I) is desired as a single isomer, it may be obtained either by resolution of the final product or by stereospecific synthesis from isomerically pure starting material or any convenient intermediate.

Resolution of the final product or an intermediate or starting material therefore may be effected by any suitable method known in the art: see, for example, Stereochemistry

of Carbon Compounds, by E.L. Eliel (McGraw Hill, 1962) and Tables of Resolving Agents, by S.H. Wilen.

The present invention is also directed to a novel methodology to prepare (-)3'-thia-2',3'-dideoxycytidine or (-)5-fluoro-3'-thia-2',3'-dideoxycytidine from (±)-3'-thia-2',3'-dideoxycytidine or (±)5-fluoro-3'-thia-2',3'-dideoxycytidine, respectively. The method takes advantage of the stereospecificity of the action of deoxycytidine deaminase (from either mammalian or bacteria sources) which converts deoxycytidine to deoxyuridine, and the separation of 3'-thia-2',3'-dideoxycytidine and SddU. Preferably, the deamination is carried out at 37°C for 16 hours.

(-)3'-Thia-2',3'-dideoxycytidine, (+)3'-thia-2',3'-dideoxycytidine and (±)3'-thia-2',3'-dideoxycytidine were examined for their HBV effect. The ID_{50} of (+)3'-thia-2',3'-dideoxycytidine, (±)3'-thia-2',3'-dideoxycytidine and (-)3'-thia-2',3'-dideoxycytidine against HBV were found to be approximately > 0.5 μm, 0.1 μm and 0.02 μm respectively, which indicates that (-)3'-thia-2',3'-dideoxycytidine is the primary form responsible for the anti HBV effect.

Cytotoxicity studies using CEM cells and dialyzed serum showed that (+)3'-thia-2',3'-dideoxycytidine is more toxic than (±)3'-thia-2',3'-dideoxycytidine, indicating that the cytotoxicity of (±)3'-thia-2',3'-dideoxycytidine observed previously is primary due to the (+)3'-thia-2',3'-dideoxycytidine, thus the therapeutic index of (-)3'-thia-2',3'-dideoxycytidine against HBV should be much better than that of (±)3'-thia-2',3'-dideoxycytidine or (+)3'-thia-2',3'-dideoxycytidine.

It was unexpected that the (-) forms of 3'-thia-2',3'-dideoxycytidine and 5-fluoro-3'-thia-2',3'-dideoxycytidine

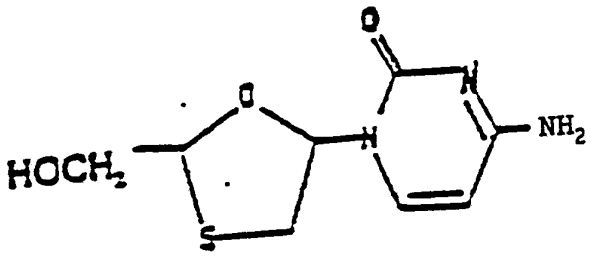
would be more active than the respective (+) forms, since the naturally existing sugar moieties have the (+) configuration.

Without wishing to be bound by any particular theory of operability, it is possible that virus DNA polymerase is able to interact with the unnatural (-)- configuration. It can be expected that the therapeutic index of (-)3'-thia-2',3'-dideoxycytidine should be better than the (+)- or (±)-form of 3'-thia-2',3'-dideoxycytidine or its analogues.

The invention will be further described by the following examples which are not intended to limit the invention in any way.

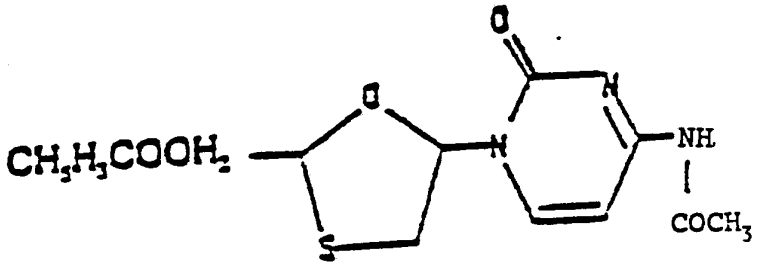
EXAMPLES

Example 1: Cis-and trans-2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolanes



(XII)

(a) Trans-: 375 mg of the trans-(XI)



(XI)

was dissolved in 100 ml of methanolic ammonia at 24°C and after stirring for 16 hours, the solvent was removed in vacuo and the residue crystallized with ether. It was recrystallized from ethanol-ether to yield 174 mg of pure product, m.p.>220° (dec). It was characterized by ^{1}H and ^{13}C NMR.

^{1}H NMR δ (ppm in DMSO-d_6);
7.57 (d. 1H; C_6-H)
7.18 (d. 2H; C_4-$N\underline{H}_2$)
6.30 (dd. 1H; C_5-$\underline{H}$)
5.68 (d. 1H; C_5-$\underline{H}$)
5.48 (t, 1H; C_2-$\underline{H}$)
5.18 (t, 1H; C_2-CH_2-$O\underline{H}$)
3.45 (m. 3H; C_2-$C\underline{H}_2OH$ +$C_4\underline{H}$)
3.06 (dd. 1H; C_4-$\underline{H}$)
U.V.: (CH_3OH) λ max: 270 mm

^{3}C NMR (DMSO-d_6, Varian XL-300); δ in ppm:

C_2	C_4	C_5	C_6	C_5	C_4	C_2	CH_2OH
154.71	165.70	93.47	140.95	87.77	36.14	86.80	64.71

(b) Cis-: treating 375 mg. of cis-(XII) by the same preceding procedure led to 165 mg of pure product after recrystallization from ethanol-ether, m.p. 171-173°. It was characterized by ^{1}H and ^{3}C NMR.

^{1}H NMR: δ(ppm in DMSO-d_6):
7.80 (d, 1H; C_6-H)
7.20 (d,2H; C_4-NH_2)
6.18 (t, 1H; C_5-H)
5.70 (d, 1H; C_5-H)
5.14 (t, 1H; C_2-CH_2OH)
3.71 (m, 2H; C_2-CH_2OH)
3.40 (dd, 1H; C_4-H)
2.99 (dd, 1H; C_4-H).
U.V.: (CH_3OH) δ max: 270 nm

Example 2: Preparation of Bis-Pivaloyl Protected Cis-2-Buten-1,4-Diol

A 10L, 3-neck flask was flame dried under vacuum and filled with argon. A positive flow of argon was maintained while the flask was fitted with a dried mechanical stirrer and charged with 100 g (0.15 eq) of DMAP, 3200 ml (39.7 moles, 7 eq) of anhydrous pyridine, and 500 g (5.67 moles, 1.00 eq) of cis-2-butene-1,4-diol. The flask was fitted with a septum. The contents were stirred at 0°C under continuous flow of argon.

1500 ml (2.15 eq) of pivaloyl chloride was added slowly, via a cannula, (in 100-200 ml portions) maintaining a low temperature and limiting gas evolution. The reaction mixture was allowed to stir for an additional hour at 0°C under argon, and was subsequently quenched by the addition of crushed ice.

The solution was decanted from the solids and the pyridine was evaporated in vacuo. The remaining material was dissolved in ether and washed once with saturated $CuSO_4$ solution, twice with saturated $NaHCO_3$ solution and twice with water. The ethereal solution was dried over $MgSO_4$, filtered, evaporated, and placed on a vacuum pump overnight.

The solid residue was dissolved in water and the resulting solution was extracted twice with ether. The ether solution was washed, dried over $MgSO_4$, filtered, evaporated, and further dried in vacuo.

Product mol. wt.	=	256.341 ($C_{14}H_{24}O_4$)
Theoretical yield	=	1,453 g
Actual yield	=	1,439 g
% yield	=	99.0%

Example 3: Coupling of Pivalate Acetate with Bis(silylated)-5-Fluoro-Cytosine

An appropriate size dry flask was charged with 199 ml methylene chloride (dried in a still) and 6.61g (corresponding to 0.0224 mols pure acetate, 1 eq) of an 88.841% (by GC analysis) of acetate under nitrogen. A second dry appropriate size flask was charged with 24 ml methylene chloride (dried in a still) and 7.67 g (0.0278 mols, 1.25 eq) of bis-silylated-5-fluorocytosine under nitrogen. After cooling the fluorocytosine mixture to 0°C, a 1.0M solution of Lewis acid in methylene chloride was added via a syringe. This mixture was then brought up to room temperature and subsequently cannulated into the acetate mixture over a 20 minute period. The reaction was monitored by thin layer chromatography using an anisaldehyde stain (anisaldehyde stain: 5% anisaldehyde, 5% concentrated H_2SO_4, 90% absolute ethanol) for visualization; disappearance of starting materials using hexane: ether, 4:1 indicated completion (45 minutes). The yellow mixture was quenched by diluting with 250 ml methylene chloride (1.5 times the total reaction solvent volume) then 47 ml concentrated ammonium hydroxide was added slowly with the addition of ice pieces to control temperature. The solution separated into two layers, the top aqueous layer which

contains some solid and the bottom organic layer which is a yellow solution. The organic layer was divided into three portions. Each portion was filtered through 1 inch of silica gel using a 300 ml fritted funnel (fresh silica gel for each portion). The silica gel layers were then washed with 6:1 ethyl acetate: absolute ethanol. The organic layers were combined and the solvents were evaporated by rotary evaporation. The product was precipitated out by adding ether and a drop or two of methylene chloride.

Filtration of the product and subsequent drying in vacuo gave a 51% pure product yield (100% β). Repeating the precipitation process allowed for the isolation of a second crop of material, bringing the total yield to 59%. The solid is a white powder.

The reaction can be performed using two different methods:

(1) Adding a Lewis acid to an acetate/silylated base mixture.

(2) Pre-mixing a Lewis acid and silylated base and adding to this mixture the acetate.

In order to obtain reasonable results, the acetate must be at least 80% pure by GC. An amount of 92.1g of an oil containing 85.3% of acetate by GC, and 64.62g of bis(trimethylsilyl)-5-fluorocytosine were dissolved in 1270 ml of dry CH_2Cl_2, all under an argon atmosphere. Next, 430 ml of a 1M Lewis acid/CH_2Cl_2 solution was added in 60 ml portions over 25 minutes. The resulting mixture was stirred for an additional 90 minutes, during which time the color changed from yellow to orange, and finally turning brown. This mixture was then diluted with 2000 ml of CH_2Cl_2 and

quenched slowly with 400 ml of concentrated NH_4OH (small amounts of ice were added to regulate the exothermicity). Upon quenching, a precipitate formed, gradually the cloudy solution separated into two layers; an aqueous layer containing some suspended solid (upper) and an organic layer (lower).

The quenched mixture was then filtered through 1-2 inches of silica gel (using a 3000 ml fritted funnel). During this process, the silica was changed 3 times due to hydration. All silica gel layers were then washed 4 times with 700 ml of a 6:1 EtoAc/EtOH mixture. The solvent was then evaporated from both the CH_2Cl_2 and EtOAc layers in vacuo.

The solid residue from the CH_2Cl_2 layer was partially dissolved with Et_2O, filtered, and the undissolved solid was washed with additional ether to give 44.46g (49%) of a white solid. The same procedure for the residue from the EtOAc layer resulted in 21.76g (24%) of an off-white powder.

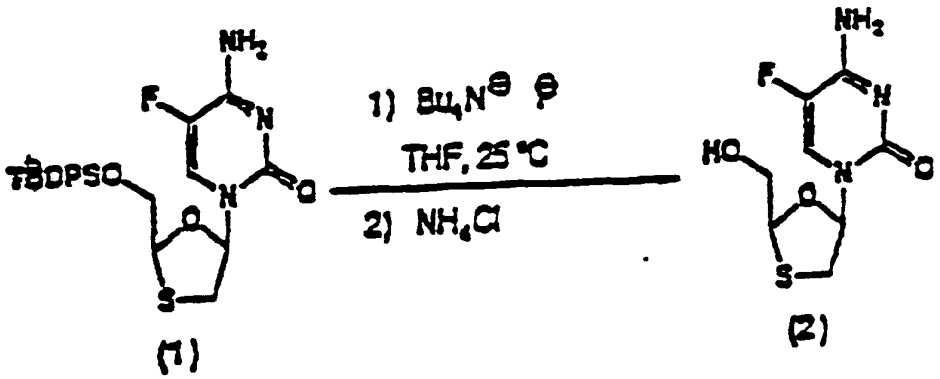
Example 4: Monitored Preparation of 2-t-Butyldiphenylsilyloxymethyl-5-Oxo-1,3-Oxathiolane

Silylated glycolaldehyde (141.41 g, 0.474 mol) is dissolved in toluene (2200 ml) in a three neck, 3000 ml round bottom flask. The flask was equipped with a stir bar, glass stopper, rubber septa, and a Dean-Stark trap to remove water during the reflux. Thioglycolic acid (33.93 ml, 0.488 mol) was added to the solution, and then heat was applied. The reaction usually takes approximately two hours to go to completion, and can be monitored by TLC (3:1 hexane:ether). By TLC the aldehyde (Rf approximately 0.3) appears just below the lactone product using a UV lamp for visualization, and when its trailing "tail" disappears, the reflux can be

stopped. GC analysis (30 m SPB-5 on fused silica cap. col) program 80°C/5 minutes -- 10°C/minutes -- 310°C is performed. The aldehyde peak appears at approximately 22.13 minutes, the lactone at approximately 27.94 minutes. This can be used to determine the final amount of aldehyde left in the reaction mixture.

When the amount of aldehyde left is negligible, cool the solution to room temperature and transfer to a separatory funnel. Wash two times with water, then extract the water layers with ether to remove residual product. Wash the combined organic layers with water once more, then dry over $MgSO_4$, filter, and concentrate. A colorless oil results, which will almost all solidify under vacuum (usually left on pump for two days).

Example 5: Standardized Procedure for the Desilylation of 1"-O-(tert-Butyldiphenylsilyl)-3'-thia-5-fluorocytidine to 5-fluoro-3'-thia-2',3-dideoxycytidine



(1) (2)

Procedure: 1"-O-(tert-Butyldiphenylsilyl)-3'-thia-5-fluorocytidine (1) 44.46 g; 91.54 mol) was dissolved in 250 mL of dry THF. Tetrabutylammonium fluoride (105 mL; 105

mmol) was added dropwise over a period of 3-5 minutes as a 1.0 M solution in THF. The progress of the reaction was monitored by TLC using 6:1 EtOAc-EtOH; visualization was accomplished by UV as well as staining with PMA following by charring. TLC analysis of the reaction mixture showed four spots: at a baseline; at R_f0.3 (corresponding to (1)); oblong-shaped spot); at R_f 0.75 (corresponding to (2)); at the solvent front (presumably TBDPS-F). The reaction mixture was stirred at room temperature; all the starting material was consumed after 45 minutes. The mixture was treated with 20 mL of saturated NH_4Cl followed by stirring for 1 hour. It was then filtered through a bed of silica gel (3 inch depth in a 350 mL fritted funnel); the silica gel was washed with 600 mL of 2:1 EtOAc-EtOH. The filtrate was reduced to a volume of approximately 100 mL by rotary evaporation during which precipitation of a white solid was observed. Filtration of the mixture gave a white powder which was a mixture of the desired product contaminated with ammonium salts. The crude solid was recrystallized from EtOH-CH_2Cl_2 to give 18.15 g (80% yield) of product. A second crop (2.09 g; 9% yield) of crystals was obtained, giving a total of 20.24 g (89% total yield) of 3'-thia-5-fluorocytidine (2): ^{1}H-NMR (DMSO-d_6; 300 MHz) 8.20 (d,J = 7.3 Hz, 1H), 7.82 (br s, 1H), 7.58 (br s, 1H), 6.15-6.12 (m, 1H), 5.41 (t,J = 5.7 Hz, 1H), 5.18 (m, 1H), 3.81-3.69 (m, 2H), 3.44 (AB dd, J = 5.4 Hz, J = 5.4 Hz, J = 11.8 Hz, 1H), 3.12 (AB dd, J = 4.3 Hz, J = 4.3 Hz, J = 11.8 Hz, 1H); α-isomer: ^{1}H-NMR (DMSO-d_6; 300 MHz) 7.83 (br d, J = 7.0 Hz, 2H), 7.56 (br s, 1H), 6.29-6.27 (m, 1H), 5.59 (t, J = 5.1 Hz, 1H), 5.19-5.16 (m, 1H), 3.57-3.38 (m, 2H), 3.17-3.12 (m, 2H).

The ratio of α:ß-isomers present may be more accurately determined by HPLC analysis of the product on a Rainin Dynamax Phenyl column using a 97 : $3H_2O$-CH_3CN isocratic

solvent system. The analysis was performed on a Shimazu LC-6A liquid chromatography system fitted with a Rainin Dynamax Phenyl column (column length: 25 cm; internal diameter: 4.6 mm; particle size 8 μm; pore size: 6Å) and linked to a UV-vis spectrophotometric detector (λ_{obs} = 265 nm). Elution was accomplished using an isocratic solvent system of 97.3 water-CH_3CN at a flow rate of 0.8 mL/minute. Under these conditions the β-isomer elutes first with a retention time (R_T) = 45.5 minutes while the α-isomer has R_T = 54.00 minutes.

Example 6: Diprotection of 1,4-Butenediol with t-Butyl Diphenylsilyl Chloride

A dry appropriate size flask was charged with 8.20 ml diol (0.996 mols, 1 eq), 2.44g DMAP (0.020 mols, 0.2 eq), and 500 ml methylene chloride (0.2 molar solution; can be done at elevated concentrations up to 0.5 molar). This solution was reduced in temperature to 0°C then 41.8 ml triethyl amine (0.300 mols, 3 eq) was added via addition funnel. The mixture needed to be shaken by hand occasionally due to viscosity and was stirred for three hours (monitoring by thin layer chromatography (4:1 hexane; ether; UV lamp for visualization) for completion. The reaction mixture was concentrated by rotary evaporation. The residue was taken up in ether and washed twice with 10% hydrochloric acid (HCl), twice with saturated aqueous sodium bicarbonate solution, and once with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated via rotary evaporation to yield 57.26 g of diprotected product.

2',3'-Dideoxy-5-fluoro-3'-thiauridine: ^1H NMR (DMSO-d^6) 11.89 (1H, broad, NH), 8.33 (1H, d, H_6, J = 7.5 Hz), 6.15 (1H, t, H, J = 3.9 Hz), 5.44 (1 H, t, OH, J = 5.7 Hz) 5.19 (1H, t, H_4, J = 3.6 Hz), 3.75 (2H, m, $2H_5$), 3.43 (1H, dd,

$1H_2$, J = 5.7 and 12.0 Hz), 3.25 (1H, dd, $1H_2$, J = 4.2 & 12.0 Hz); mp 158-159°C; Anal. Calc. for $C_8H_9O_4N_2SF$:$C_1$38.71; H, 3.65; N, 11.29; S, 12.92.
Found: C, 38.79: H, 3.68; N, 11.23; S, 12.82.

2',3'-Dideoxy-5-fluoro-3'-thiacytidine: ^1H NMR (DMSO d^6) 8.18 (1H, d, H_6, J = 8.4 Hz) 7.81 and 7.57 (2H, broad NH2), 6.12 (1H, dd, H_1, J = 5.7 and 4.2 Hz), 5.40 (1H, t, OH, J = 5.7 Hz), 5.17 (1H, t, H_4, J = 3.6 Hz), 3.74 (2H, M, $2H_5$), 3.41 (1H, dd, 1 H_2, J = 5.7 and 11.7 Hz), 3.11 (1H, dd, $1H_2$, J = 4.2 and 11.7 Hz); mp 195-196°C; Anal. Calc. for $C_8H_{10}O_3N_3SF$:C, 38.86; H, 4.08; N, 17.00; S, 12.97. Found: C, 38.97; H, 4.07; N, 16.93; S, 12.89.

Example 7: In Vitro Assay for Antiviral Activity

2.2.15 cells were inoculated at a density of 3 X 10^5 cells/ 5ml in 25 cm^2 flask. Drugs were added to the medium 3 days after the inoculation. Cells were grown in the presence of drugs for 12 days With changes of medium every 3 other days. At end of the incubation, the medium was centrifuged (10 minutes, 2,000 X g) and the supernatant was subjected to a final concentration of 10% (wt/vol) PEG 8,000. The virus was pelleted (10 minutes 10,000 X g). The pellet was resuspended at 1/100th the original volume in TNE buffer (10 mM Tris pH 7.5, 100 mM NaCl, 1 mM EDTA). The suspension was adjusted to 1 % SDS and 0.5 mg/ml proteinase K and incubated for 2 hours at 55°C. The digest was extracted with phenol, chloroform and the DNA was precipitated with ethanol. The DNA pellet was dissolved in TE_{80} (10 mM Tris pH 8.0, 1 mM EDTA) and electrophoresed in a 0.8% agarose gel, followed by blotting onto Hybond-N membrane (Amersham). The filter was hybridized with ^{32}P-labeled HBV DNA probe, washed with 2 X SSC containing 0.2 % SDS at room temperature for 1 hour, 0.1

X SSC containing 0.2 % SDS at 55°C for 1/2 hour and autoradiographed.

Example 8: Isolation and Characterization of DNA

Drug-treated cells were lysed with a buffer containing 10 mM Tris pH 7.5, 5 mM EDTA, 150 mM NaCl and 1% SDS. The cell lysate was digested with 100 μg/ml proteinase K at 55°C for at least 2 hours and deproteinized by extraction with phenol. Nucleic acids were precipitated with 2 volumes of ethanol. The pellet of nucleic acid was redissolved in 10 mM Tris pH 8.0, 1 mM EDTA followed by 100 μg per ml RNase treatment at 37°C for 1 hour. Concentrated ammonium acetate was added to the aqueous phase to yield a final 0.4 M ammonium acetate solution. The nucleic acids were precipitated with ethanol.

Example 9: Isolation of RNA

Total cellular RNA was isolated according to Chomczyndki et al. (Chomczynsk, P., and Sacchi, N., "Single Step Method for RNA Isolation by Acid Guanidinium Thiocyanate- Phenol- Chloroform Extraction," Analytical Biochemistry, 162: 156-159, 1987). The RNA (20 μg per lane) was electrophoresed through 1% agarose gel containing 1.1 M formaldehyde and transferred to Hybond-N membrane. The immobilized RNA was hybridized with ^{32}P-labeled HBV DNA probe and the membrane was autoradiographed as described above.

Example 10: Cytotoxicity

CEM (T lymphoblastoid cells) cells were inoculated in 5 ml RPMI 1640 supplemented with 5% fetal bovine serum at a

concentration of 2 X 10^3 cells per ml. The cells were incubated with different concentrations of compounds for 4 days. At day 4, the cell number was determined by a coulter counter or a hemocytometer.

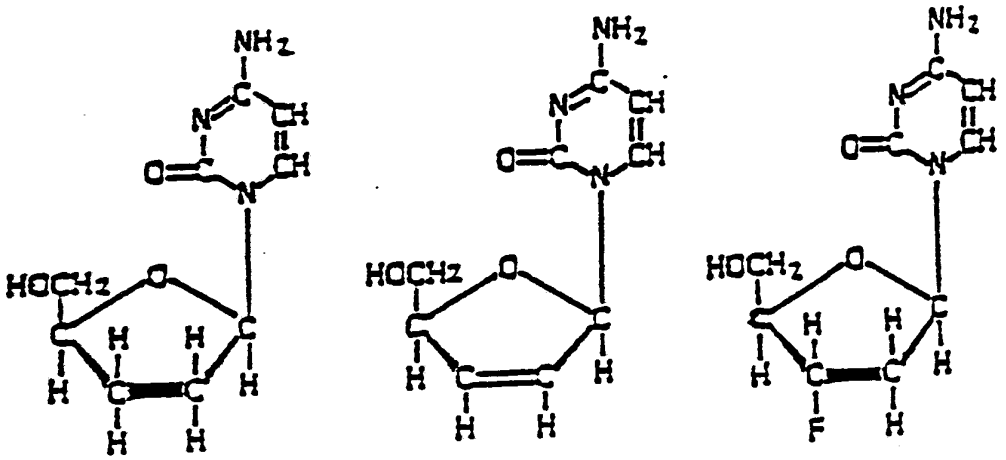
Example 11: Determination of mtDNA Content by Quick Blot Procedure

The CEM cells (5 X 10^4) cells were collected and freeze-thawed three times. The cell lysate was incubated with RNase (10 μg/ml) at 37°C for 30 minutes. The sample was treated with proteinase K (100 μg/ml) at 55°C for 1 hour. A 0.8 volume of supersaturated Nal (2.5 gNaI in 1 ml hot water) was added to the sample and heated at 90°C for 10 minutes. The DNA was immobilized on nitrocellulose paper by using a slot blot apparatus (Schleicher & Schuell, Keene, NH). The mtDNA on the nitrocellulose paper was detected with a mtDNA specific probe as described previously (Chen, C.H., Cheng, Y.C., "Delayed Cytotoxicity and Selective Loss of Mitochondria DNA in Cells Treated with the Anti-Human Immunodeficiency Virus Compound 2',3'-Dideoxycytidine," J. Biol. Chem. 264: 11934-11937, 1989).

Example 12: Comparative Potency of Deoxycytidine Analogs as Inhibitors of HBV In Vitro

2.2.15 cell lines was used to evaluate the antiviral activities of modified dideoxycytidine analogs -- ddC (obtained from Pharmacia, Inc.), D4C, 3'-fluoro-2',3'-dideoxycytidine (3'-FddC), 5-fluoro-3'-thia-2'3-dideoxycytidine (5-FSddC) and 3'-thia-2',3'-dideoxycytidine (SddC).

The formulas for ddC, d4C and 3'-FddC are as follows:



ddC D4C 3'-FddC

The antiviral effects were measured by analysis of extracellular HBV DNA (Fig.1). The experiment revealed that the amount of extracellular HBV DNA decreased in a dose dependent manner. The inhibitory concentration for a 50% decrease in viral replication ($HBID_{50}$) of these compounds are presented in Table 1. At concentration of 2 μM, both 5-fluoro-3'-thia-2',3-dideoxycytidine and 3'-thia 2',3'-dideoxycytidine completely inhibited the replication of HBV, approximately 50% inhibition by 3'-FddC, whereas neither ddC nor D4C had any impact effect. Episomal HBV DNAs in 2.2.15 cells treated with different concentrations of 5-fluoro-3'-thia-2',3'-dideoxycytidine and 3'-thia-2',3'-dideoxycytidine were also traced. Cellular DNAs were digested with Hind III that does not cleave within HBV genome, and subjected to Southern analysis using P^{32}-labeled HBV DNA as a probe. The chromosomally integrated HBV DNA genome and the episomal DNA can be separated in the gel and can be differentially quantified. While episomal DNA decreased in a dose dependent manner as extracellular HBV DNAs, both the amount and the

restriction enzyme pattern of the chromosomally integrated HBV genome were unaltered (Fig.2).

Example 13: Comparative Cytotoxicity and Effect on Mitochondria DNA Content of Deoxycytidine Analogs

ED_{50}s (concentration of compounds which caused a 50% reduction in cell density) are shown in Table 1. Mitochondria DNA content was measured by slot blot hybridization analysis. MtID50s (concentration of compounds which caused a 50% reduction in mitochondria DNA content) are also shown in Table 1. Both 5-fluoro-3'-thia-2',3-dideoxycytidine and 3'-thia 2',3'-dideoxycytidine inhibited HBV replication at concentrations hundreds or thousands fold lower than concentrations which induced cytotoxicity in CEM cells. Moreover, at concentrations which caused cessation of HBV replication, no effect on mitochondria DNA synthesis was observed. This contrasts with ddC (a potent polymerase gamma inhibitor) which had a $HBID_{50}$ of 3.8 μM but a low $MtID_{50}$ at 0.022 μM. D4C had a lower value of $MtID_{50}$ (2.0 μM) than $HBID_{50}$ (2.4 μM). Thus, D4C would be expected to have a mitochondria effect before the anti-HBV effect was achieved as the same phenomenon observed in treatment with ddC. 3'-FddC had a greater selective index than both ddC and D4C. However, the ED_{50} versus the $MTID_{50}$ ratio is about the same as ddC, suggesting 3'-FddC is not the drug of choice either.

Table 1: Comparative Potencies of Dideoxycytidine Analogues as Monitored by Anti-HBV, Cytotoxicity and Mitochrondria DNA Effect

Compounds	$HBID_{50}$[1] (μM)	$MTID_{50}$[2] (μM)	ED_{50}[3] (μM)	S.I.[4]	$ED_{50}/MtID_{50}$
ddC	3.8	0.022	10	2.6	454
D4C	2.4	2	22	9.2	11
3'-FddC	1.6	0.11	45	28.1	409
3'thia-2',3'-dideoxycytidine	0.05	47	37	740	0.79
5-fluoro-3'-thia-2', 3-dideoxycytidine	0.1	>200	>200	>2000	NA

1: concentration which caused a 50% reduction in HBV replication

2: concentration which caused a 50% reduction in mitochondria DNA content

3: concentration which caused a 50% reduction in cell density

4: Selective Index ($ED_{50}/HBID_{50}$)

.: nonapplicable

Example 14: Reversibility of 5-fluoro-3'-thia-2',3-dideoxycytidine and 3'thia-2',3'-dideoxycytidine

To determine whether the antiviral effect was reversible, 2.2.15 cells that were treated with a 2.0 μM concentration of 5-fluoro-3'-thia-2',3-dideoxycytidine and 3'-thia-2',3'-dideoxycytidine for 12 days were incubated for additional 6 or 12 days in the absence of the compound. After 6 days of

drug-free incubation, HBV DNA could again be identified extracellularly (Fig.3), though not as much as those in control untreated cells. After 12 days of drug-free incubation, both extracellular (Fig.3) and intracellular episomal HBV DNAs (Fig.2) bounced back.

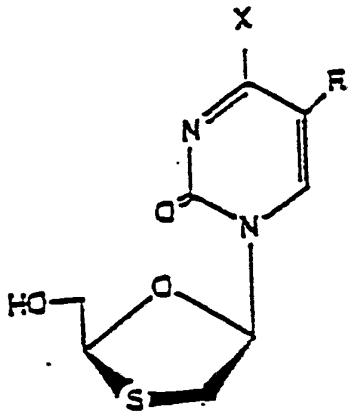
Example 15: Detection of HBV-specific RNAs in Both Control and Drug Treated 2.2.15 Cells.

Northern blot analysis was performed to analyze HBV RNA transcripts. Three major transcripts of approximately 3.5, 2.5, and 2.1 Kb were detected in the total cellular RNA extract. The HBV specific transcripts were not affected at all by 2.0 μM 5-fluoro-3'-thia-2',3'-dideoxycytidine and 3'-thia-2',3'-dideoxycytidine treatment (Fig. 4).

Example 16: Comparative Potency of Pyrimidine 3'-thia 2',3'-dideoxynucleoside Analogs as Inhibitors of HBV In Vitro

Various analogs (Table 2) of 3'-thia-2',3'-dideoxycytidine were tested in 2.2.15 cells for anti-HBV activity. At 1.0 μM, none of the 3'-thia 2',3'-dideoxyuridine analogs was active against HBV replication (Fig.5). Among the 3'-thia 2',3'-dideoxycytidine analogs tested, both 3'-thia-2',3'-dideoxycytidine (R_5=H) and 5-fluoro-3'-thia-2',3-dideoxycytidine (R_5=F) had the most potent inhibitory effect on HBV replication. At 1.0 μM, 5-Br, 5-Cl, and 5-CH_3 analogs are not active, whereas the 5-I analog reduced the HBV production by at least 2 fold.

TABLE 2



	X	R
	OH	H
	OH	Me
	OH	F
	OH	Cl
	OH	Br
	OH	I
3'-thia-2', 3'-dideoxy-cytidine	NH_2	H
	NH_2	Me
5-fluoro-3'-thia-2',3'-dideoxy-cytidine	NH_2	F
	NH_2	Cl
	NH_2	Br
	NH_2	I

Example 17

Preparation of (-)3'-thia-2',3'-dideoxycytidine from (±)-3'-thia-2',3'-dideoxycytidine

Since the (-)3'-thia-2',3'-dideoxycytidine was not available by chemical synthesis and the (+)-3'-thia-2',3'-dideoxycytidine and (-)3'-thia-2',3'-dideoxycytidine cannot be separated by conventional methods, applicants developed a novel methodology to separate (-)3'-thia-2',3'-dideoxycytidine from (+)3'-thia-2',3'-dideoxycytidine by taking the advantage of the deoxycytidine (structure <1>) deaminase (from either bacteria or mammalian sources). (+)3'-Thia-2',3'-dideoxycytidine can be selectively deaminated by dCdR (deoxycytidines deaminase), based on HPLC retention time and UV spectrum and leave (-)3'-thia-2',3'-dideoxycytidine intact from (±)3'-thia-2',3'-dideoxycytidine mixture. The reaction mixture which contains (+)-3'-thia-2',3'-dideoxycytidine (retention time 8.46 minutes, UV_{max} 260 nm) and (-)3'-thia-2',3'-dideoxycytidine (retention time 7.05 minutes, UV_{max} 270 nm) can then be separated by HPLC (Fig. 6A and Fig. 6B, using 100 mM NH_4Ac to achieve better separation). A control reaction which has only (+)3'-thia-2',3'-dideoxycytidine proved that (+)3'-thia-2',3'-dideoxycytidine is the substrate of CdR deaminase and can be almost 100% deaminated (Fig. 7A and Fig. 7B). (±)3'-Thia-2',3'-dideoxycytidine can be deaminated only up to approximately 50%; no further deamination has been observed. After HPLC separation, (-)3'-Thia-2',3'-dideoxycytidine was resubjected to deaminase and showed no sign of deamination at all (Figs. 8A and 8B). α-3'-Thia-2',3'-dideoxycytidine has a different retention time from (+)3'-thia-2',3'-dideoxycytidine on HPLC (α-3'-thia-2',3'-dideoxycytidine has a retention time of approximately 6.9 minutes compared with (+)3'-thia-2',3'-dideoxycytidine which has a retention time of approximately 8.3 minutes using no

salt solvent system under the same condition as the one used in the case of (+)3'-thia-2',3'-dideoxycytidine and (-)3'-thia-2',3'-dideoxycytidine). However, no deamination occurred (Fig. 9A and Fig. 7B) under the same condition that (+)3'-thia-2',3'-dideoxycytidine was completely deaminated.

A similar pattern of deamination of (±)5-fluoro-3'-thia-2',3'-dideoxycytidine was also observed and the product, (+)5-fluoro-3'-thia-2',3'-dideoxyuridine and (-)5-fluoro-3'-thia-2',3'-dideoxycytidine was confirmed using the chemically synthesized 5-fluoro-3'-thia-2',3'-dideoxyuridine as a standard.

Example 18: Assay Condition (HPLC Analysis)

The reaction mixture contained 25 mM Tris-HCl, pH 8.0, 0.1 mM of 3'-thia-2',3'-dideoxycytidine and 0.2 unit of human cytidine deaminase (unit definition = the amount of enzyme which converts 1 n mole of cytidine to uridine per minute at 37°C) in a total volume of 50 µl. After each time point, 100 µl of acetonitrile were added to the reaction mixture and agitated. The precipitated proteins were removed by centrifugation, and the supernatent was lyophilized to dryness. The samples were reconstituted with 200 µl of HPLC mobile phase buffer and analyzed on an Alltech RP-18 column. 3'-Thia-2',3'-dideoxycytidine and SddU were detected at a UV absorption wavelength of 270 nm. The mobile phase was 10% acetonitrile/water unless specified otherwise, and the flow rate was 0.8 ml/minutes.

Example 19: In Vitro Assay for Antiviral Activity

The 2.2.15 cells (clonal cells derived from Hep G2 cells that were transfected with a plasmid containing HBV DNA) that secrete hepatitis B virions were used. The 2.2.15 cells were

maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum. Cells were incubated at 37°C in a moist atmosphere containing 5% CO_2 in air. The 2.2.15 cells were inoculated at a density of 3 X 10^5 cells/ 5 ml in 25 cm^2 flask. The compounds studied were added to the medium three days after inoculation. Cells were grown in the presence of drugs for 12 days with changes of medium every three days. After incubation, the medium was centrifuged (10 minutes, 2,000 X g) and polyethylene glycol (M_r 8,000) was added to the supernatant to a final concentration of 10% (wt/vol.) The virus was pelleted (10 minutes 10,000 X g). The pellet was resuspended at 1/100th the original volume in TNE buffer (10 mM Tris pH 7.5, 100 mM NaCl, 1 mM EDTA). The suspension was adjusted to 1% SDS and 0.5 mg/ml proteinase K and incubated for 2 hours at 55°C. The digest was extracted with phenol-chloroform and the DNA was precipitated with ethanol. The DNA pellet was dissolved in TE_{80} (10 mM Tris HCl pH 8.0, 1 mM EDTA) and then electrophorsed in a 0.8% agarose gel followed by blotting onto Hybond-N membrane (Amersham, Arlington, Illinois). The filter was hybridized with ^{32}P-labeled HBV DNA (Bam HI insert from plasmid Pam6 (American Type Culture Collection, Rockville, Maryland) probe, washed with 2 X SSC containing 0.2% SDS at room temperature for 1 hour, 0.1 X SSC containing 0.2% SDS at 55°C for 1/2 hour and then autoradiographed. The intensity of the autoradiographic bands was quantitated by a scanning densitometer. The amount of HBV-specific DNAs was similar in separate experiments performed in duplicate. $HBID_{50}$ was defined as the drug concentration that inhibited HBV viral DNA yield in the medium by 50%. The values were obtained by plotting percentage inhibition compared with control versus the drug concentration.

Example 20:

Comparative Potency of (+)3'-thia-2',3'-dideoxycytidine, (-)3'-thia-2',3'-dideoxycytidine and (±)3'-thia-2',3'-dideoxycytidine as Inhibitors of HBV In Vitro

The 2.2.15 cell line was used to evaluate the antiviral activities of (+)3'-thia-2',3'-dideoxycytidine, (-)3'-thia-2',3'-dideoxycytidine and (±)3'-thia-2',3'-dideoxycytidine. The antiviral effects were measured by analysis of extracellular HBV DNA (Fig. 12). The experiment revealed that the amount of extracellular HBV DNA decreased in a dose dependent manner. At a concentration of 0.5 μm (±)3'-thia-2',3'-dideoxycytidine completely inhibited the replication of HBV, whereas there was approximately only 50% inhibition by (+)3'-thia-2',3'-dideoxycytidine. The $HBID_{50}$ of (-)3'-thia-2',3'-dideoxycytidine was estimated to be 0.02 μm which is significantly lower than that of (±)3'-thia-2',3'-dideoxycytidine ($HBID_{50}$ = 0.1 μm).

Summary of the Biological Activity

The anti-HBV effect of each of the two racemic 3'-thia-2',3'-dideoxycytidine was examined as described above. The ID_{50} of (+)3'-thia-2',3'-dideoxycytidine, (±)3'-thia-2',3'-dideoxycytidine and (-)3'-thia-2',3'-dideoxycytidine are approximately > 0.5 μm, 0.1 μm and 0.02 μm, respectively, which indicates that (-)3'-thia-2',3'-dideoxycytidine is the primary form responsible for the anti-HBV effect observed for (±)3'-thia-2',3'-dideoxycytidine. Furthermore, the (+)-form may even interfere with the (-)- form based on the observation that the (-)- form is approximately 5 fold more active than the (±) form of 3'-thia-2',3'-dideoxycytidine at the same dose (if there is no interference, it should be only 2-fold). A similar observation was also made for 5-fluoro-3'-thia-2',3'-dideoxycytidine.

Example 21: CEM Growth in 3'-thia-2',3'-dideoxycytidine

CEM were seeded at 3.5 x 10^4 cells per ml onto Corning 6-well dishes at 5 ml per well in RPMI 1640 + 5% dialized FBS + kanamycin at 100 mg/ml.

Two wells were treated for each of the following conditions:

3, 10, 30, 100 μm + SddC
3, 10, 30, 100 μm ± SddC.

Six wells were seeded as controls. These wells were untreated.

Cell number was determined on days 2, 4 and 6 after seeding. The cells were pipetted to break clumps, and then 1 ml was removed from each well and diluted into 9 ml saline. The saline + cells were pipetted well to break clumps and counted via a coutler counter.

To determine ID_{50}, cell number was plotted versus days in culture. (+)3'-thia-2',3'-dideoxycytidine was seen to be very toxic to the cells with an ID_{50} of 1.26 μm. (±)3'-thia-2',3'-dideoxycytidine was less toxic with an ID_{50} of 7.2 μm.

The cytotoxicity studies using CEM cells and dialyzed serum showed that (+)3'-thia-2',3'-dideoxycytidine is more toxic (ID_{50}: 1.3 μm) than (±)3'-thia-2',3'-dideoxycytidine (ID_{50}: 7.2 μm) suggesting that the (-)-form is less toxic than (+)-form and the (±)-form.

Examples 22-26

The numbers in parentheses in Examples 22-26 refer to the compound numbers in Scheme 1.

Example 22:
(-)-1,6-Anhydro-α-L-gulopyranose (3)

A mixture of (2) (33 g,0.127 mol) and 0.5 N HCl (330 mL, 0.165 mol) was refluxed for 20 hours and the mixture was cooled and neutralized to pH 6 by a resin (Dowex-2, HCO_3-form) with air bubbling. The resin was recycled by washing with 10% HCl, water, methanol, water and saturated $NaHCO_3$ solution. The reaction mixture was filtered and the resin was washed with water (500 mL). The combined filtrate was concentrated to dryness and dried in vacuo overnight. The residue was purified over a column (5 cm depth, silica gel, mesh, $CHCl_3$-MeOH, 10:1) to give a slightly yellow solid, which was recrystallized from absolute alcohol to give a colorless solid (3) [R_f = 0.43 ($CHCl_3$-MeOH, 5:1),7.3g, 35.52%]. The L-gulose (R_f=0.07, 11g) obtained was recycled to give (3) (5g, total yield 60%):mp 142.5-145°C; ^1H NMR(DMSO-d_6) δ 3.22-3.68(m, 4H, H-2, -3, -4 and -6a), 3.83 (d,$J_{6b,6a}$= 7.25 Hz, 1H, H_b-6), 4.22 (pseudo t, $J_{5,6a}$= 4.61 and 4.18 Hz, H, H-5), 4.46 (d, $J_{2\text{-}OH,2}$=6.59 Hz, 1H, 2-OH, exchangeable with D_2O), 4.62 (d, $J_{3\text{-}OH,3}$= 5.28 Hz, 1 H, 3-OH, exchangeable with D_2O), 5.07 (d, $J_{4\text{-}OH,4}$=4.84 Hz, 1H, 4-OH, exchangeable with D_2O), 5.20 (d, $J_{1,2}$=2.19 Hz, 1H,H-1).$[\alpha]_D^{25}$-50.011 (c, 1.61, MeOH).

Example 23:
(-)-(1'S,2S,4S)-4-(1,2-Dihydroxyethyl-1,2-O-Isopropylidene)-2-hydroxymethyl)-dioxolane (5)

To a solution of (3) (11.3 g, 0.07 mol) in methanol (350 mL) was added dropwise a solution of $NaIO_4$ (22.36 g, 0.1 mol) in water (300 mL) for 10 minutes at 0°C and the mixture was stirred mechanically for 15 minutes. $NaBH_4$(7.91 g, 0.21 mol) was added to this mixture and the reaction mixture was stirred for 10 minutes at 0°C. The white solid was filtered off and the solid was washed with methanol (300 mL). The combined filtrate was neutralized by 0.5 N HCl (~200 mL) and concentrated to dryness. The residue was dried in vacuo overnight. The syrupy residue was triturated with methanol-acetone (1:5, 1200 mL) using a mechanical stirrer (5 hours) and the white solid (1st.) was filtered off. The filtrate was concentrated to dryness and the residue was dissolved in acetone (500 mL) and followed by p-TsOH (6.63 g, 0.035 mol). After stirring for 6 hours, the mixture was neutralized by Et_3N and the solid (2nd.) was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in ethyl acetate (350 mL) and washed with water (500 mL X 2), dried ($MgSO_4$), filtered, evaporated to give crude (5) (3.6 g) as a yellowish syrup. The water layer was concentrated to dryness and dried in vacuo. The solid obtained (1st and 2nd) was combined with the dried water layer and recycled using 10% methanol-acetone (900 mL), p-TsOH (16 g, 0.084 mol) by 1 hour stirring to yield crude (5)(5.6g). The crude product obtained was purified by a dry column over silica gel (MeOH-$CHCl_3$, 1%-5%) to give (5)[R_f= 0.82($CHCl_3$-MeOH, 10:1), 8.8 g, 61.84%] as a colorless oil. ^1H NMR(DMSO-d_6) δ 1.26 and 1.32 (2 X s, 2 X 3 H, isopropylidene), 3.41 (dd, $J_{CH2OH,OH}$ = 6.04 Hz, $J_{CH2OH,2}$=3.96 Hz, 2H, CH_2OH), 3.56-4.16(m, 6H, H-4, -5, -1' and -2'), 4.82 (t, $J_{OH,CH2}$ = 6.0 Hz, 1 H, CH_2OH, exchangeable with D_2O), 4.85 (t,

$J_{2OH,CH2OH}$ = 3.96 Hz, 1H, H-2).$[\alpha]_D^{25}$-12.48 (c, 1.11, $CHCl_3$), Anal, Calcd for $C_9H_{16}O_5$:C,52.93; H,7.90. Found:C,52.95; H,7.86.

Example 24:
(+)-(1'S,2S,4S)-4-(1,2-Dihydroxymethyl-1,2-O-Isopropylidene)-2-(O-benzoyloxymethyl)-dioxolane (6)

To a solution of **(5)** (8.5 g, 0.042 mol) in pyridine-CH_2Cl_2 (1:2, 120 mL) was added dropwise benzoyl chloride (6.5 mL, 0.056 mol) at 0°C and the temperature was raised to room temperature. After stirring for 2 hours, the reaction was quenched with methanol (10 mL) and the mixture was concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2 (300 mL) and washed with water (100 mL X 2), brine, dried ($MgSO_4$), filtered, evaporated to give a yellowish syrup, which was purified by silica gel column chromatography (EtOAc-Hx, 4% -30%) to yield **(6)** [R_f = 0.45 (Hx-EtOAc, 3:1), 10.7 g, 83.4%] as a colorless oil. 1H NMR ($CDCl_3$) δ 1.35 and 1.44 (2 X s, 2 X 3H, isopropylidene) 3.3-4.35 (m 6H, H-4, -5, -1' and -2'), 4.44 (d, J=3.96 Hz, 2H, CH_2-OBz), 5.29 (t, J=3.74 Hz, 1H,H-2), 7.3-7.64, 8.02-8.18 (m, 3H, 2H, -OBz).$[\alpha]_D^{25}$+10.73(c,1.75,MeOH). Anal. Calcd for $C_{16}H_{20}O_6$:C,62.33;H,6.54. Found: C,62.39; H, 6.54.

Example 25
(+)-(1'S,2S,4S)-4-(1,2-Dihydroxyethyl)-2-(O-benzoyloxymethyl)-dioxolane (7)

The mixture of **(6)** (5.7 g. 0.018 mol) and p-TsOH (1.05 g. 0.0055 mol) in methanol (70 mL) was stirred at room temperature for 2 hours. But the reaction was not completed, so the solvent was evaporated to half volume and were added methanol (50 mL) and p-TsOH (0.7 g, 3.68 mmol). After stirring for one more hour, the reaction mixture was neutralized by Et_3N and the solvent was evaporated to dryness.

The residue was purified by silica gel column chromatography (Hx-EtOAC, 10%-33%) to give **(7)** [R_f=0.15(Hx-EtOAc, 1:1), 4.92 g, 99.2%] as a colorless syrup ^{1}H NMR (DMSO-d_6) δ 3.43 (m, 2H, H-2'), 3.67-4.1 (m, 4H, H-4, -5 and -1'), 4.32 (d, J= 3.73 Hz, 2H, CH_2-OBz), 4.60 (t,J=5.72 Hz, 2'-OH, exchangeable with D_2O), 5.23 (t, J= 3.96 Hz, 1H, H-2), 7.45-7.7,7.93-8.04 (m, 3H, 2H, -OBz), $[\alpha]_D^{25}$ + 9.16 (c,1.01, $CHCl_3$). Anal. Calcd for $C_{13}H_{16}O_6$:C,58.20; H,6.01. Found: C,58.02; H.6.04.

Example 26:
(-)-(2S,4S) and (2S,4R)-4-Acetoxy-2-(O-benzoyloxymethyl)-dioxolane **(9)**

To a solution of **(7)** (3.04 g, 0.011 mol) in CCl_4-CH_3CN (1:1, 160 mL) was added a solution of $NaIO_4$ (10.18 g, 0.048 mol) in water (120 mL) and then RuO_2·hydrate (0.02g). After the reaction mixture was stirred for 5 hours, the solid was removed by filtration over Celite and the filtrate was evaporated to 1/3 volume. The residue was dissolved in CH_2Cl_2(100 mL) and the water layer was extracted with CH_2Cl_2 (100 mL X 2). The combined organic layer was washed with brine (50 mL), dried ($MgSO_4$), filtered, evaporated to dryness and dried in vacuo for 16 hours to give crude **(8)** (2.6 g, 91%).

To a solution of crude **(8)** (2.6 , 0.01 mol) in dry THF (60 mL) were added $Pb(OAc)_4$(5.48 g, 0.0124 mol) and pyridine (0.83 mL, 0.0103 mol) under N_2 atmosphere. The mixture was stirred for 45 minutes under N_2 and the solid was removed by filtration. The solid was washed with ethyl acetate (60 mL) and the combined organic layer was evaporated to dryness. The residue was purified by silica gel column chromatography (Hx-EtOAc, 2:1) to yield **(9)** [R_f = 0.73 and 0.79 (Hx-EtOAc, 2:1), 1.9 g, 69.34%] as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.998, 2.11 (2X s, 3H, -OAc), 3.93-4.33 (m, 2H, H-5), 4.43, 4.48 (2 X

d, J= 3.73, 3.74 Hz, 2H, CH_2OBz), 5.46, 5.55 (2 X t, J= 4.18, 3.63 Hz, 1H, H-2), 6.42 (m, 1H, H-4), 7.33-7.59, 8.00-8.15 (m, 3H, 2H, -OBz). $[\alpha]_D^{25}$-12.53 (c, 1.11, $CHCl_3$). Anal. Calcd for $C_{13}H_{14}O_6$; C, 58.64; H, 5.30. Found: C, 58.78; H, 5.34.

Examples 27-29

The numbers in parentheses in Examples 27-29 refer to compound numbers in Scheme 2.

Example 27:

(-)-(2S,4S)-1-[2-(benzoyl)-1,3-dioxolan-4-yl]cytosine(2),(+)-(2S,4R)-1-[2-(benzyloxy)-1,3-dioxolan-4-yl)cytosine(3)

A mixture of N^4-acetylcytosine (1.24 g, 7.52 mmol) in dry dichloroethane (20 mL), hexamethyldisilazane (15 mL), and ammonium sulfate (cat.amount) was refluxed for 4 hours under a nitrogen atmosphere. The resulting clear solution was cooled to room temperature. To this silylated acetylcytosine a solution of **(1)** (1.0 g, 3.76 mmol) in dry dichloroethene (10 mL) and TMSOTf (1.46 mL 7.55 mmol) were added and stirred for 6 hours, then saturated $NaHCO_3$ (10 mL) was added and stirred for another 15 minutes and filtered through a Celite pad. The filtrate was evaporated and the solid was dissolved in EtOAc and washed with water and brine, dried, filtered and evaporated to give the crude product. This crude product was purified on a silica column (5% MeOH/$CHCl_3$) to yield a pure α,ß mixture of **(2)** and **(3)** (0.40 g, 30%) and the α,ß mixture of **(4)** and **(5)** (0.48 g, 40%). The mixture of **(4)** and **(5)** was reacetylated for separation, the combined α,ß mixture was separated by a long silica column (3% MeOH/$CHCl_3$) to yield **(2)** (0.414g, 30.7%) and **(3)** (0.481 g, 35.6%) as foams. These foams were triturated with MeOH to obtain white solids. **2:** UV (MeOH) λ max 298 nm; Anal. ($C_{17}H_{17}N_3O_8$) C, H, N. **3:** UV (MeOH) λ max 298 nm.

Example 28:

(-)-(2S,4S)-1-(2-Hydroxymethyl-1,3-dioxolan-4-yl)cytosine(6)

A solution of (2) (0.29g, 0.827) in MeOH/NH_3 (50 mL, saturated at 0°C) was stirred at room temperature for 10 hours. The solvent was evaporated and the crude was purified on preparative silica plates (20% MeOH/$CHCl_3$) to give an oil. This was crystallized from CH_2Cl_2/hexane to give (6) (0.136 g, 77.7%) as a white solid. UV λ max 278.0 nm (ε 11967) (pH 2), 270.0 nm (ε 774) (pH 7), 269.0 nm (ε8379) (pH 11); Anal. ($C_8H_{11}N_3O_4$)C,H,N.

Example 29:

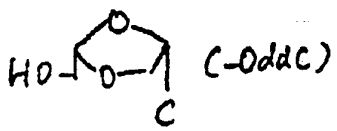
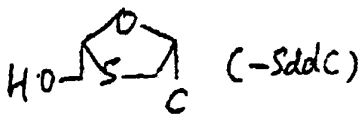
(+)-(2S,4R)-1-(2-Hydroxymethyl-1,3-dioxolan-4-yl)cytosine(7)

A solution of (2) (0.35 g, 0.991) in MeOH/NH_3 (50 mL), saturated at 0°C was stirred at room temperature for 10 hours. The solvent was evaporated and the crude was purified on preparative silica plates (20% MeOH/$CHCl_3$) to give an oil. This was crystallized from CH_2Cl_2/hexane to give (7) (0.135 g, 64.3%) as a white solid. UV λ max 278.0 nm (ε 13241) pH 2), 270.0 nm (ε 13241) (pH 2), 270.0 nm (ε 8780) (pH 7), 269.0 nm (ε 9071) (pH 11); Anal.($C_8H_{11}N_3O_4$)C, H, N.

Example 30:

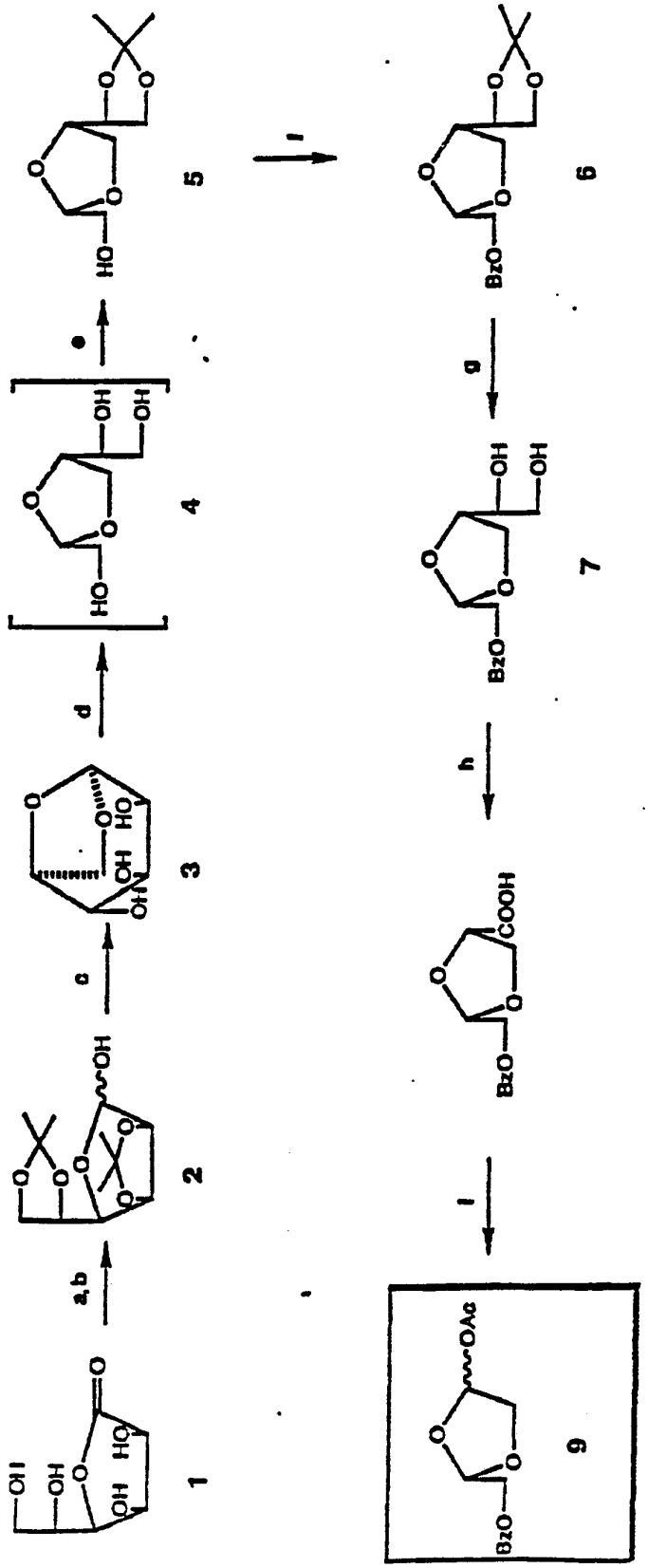
Results of Biological Testing

Compounds - OddC and - SddC were tested following the same procedure as in Example 20 using the 2.2.15 cell line.

Compound	$HBVID_{50}$	Toxicity (Anti CEM cell ID_{50})
 (-OddC)	<0.001μM	> 5μM
 (-SddC)	0.01μM	~50μM

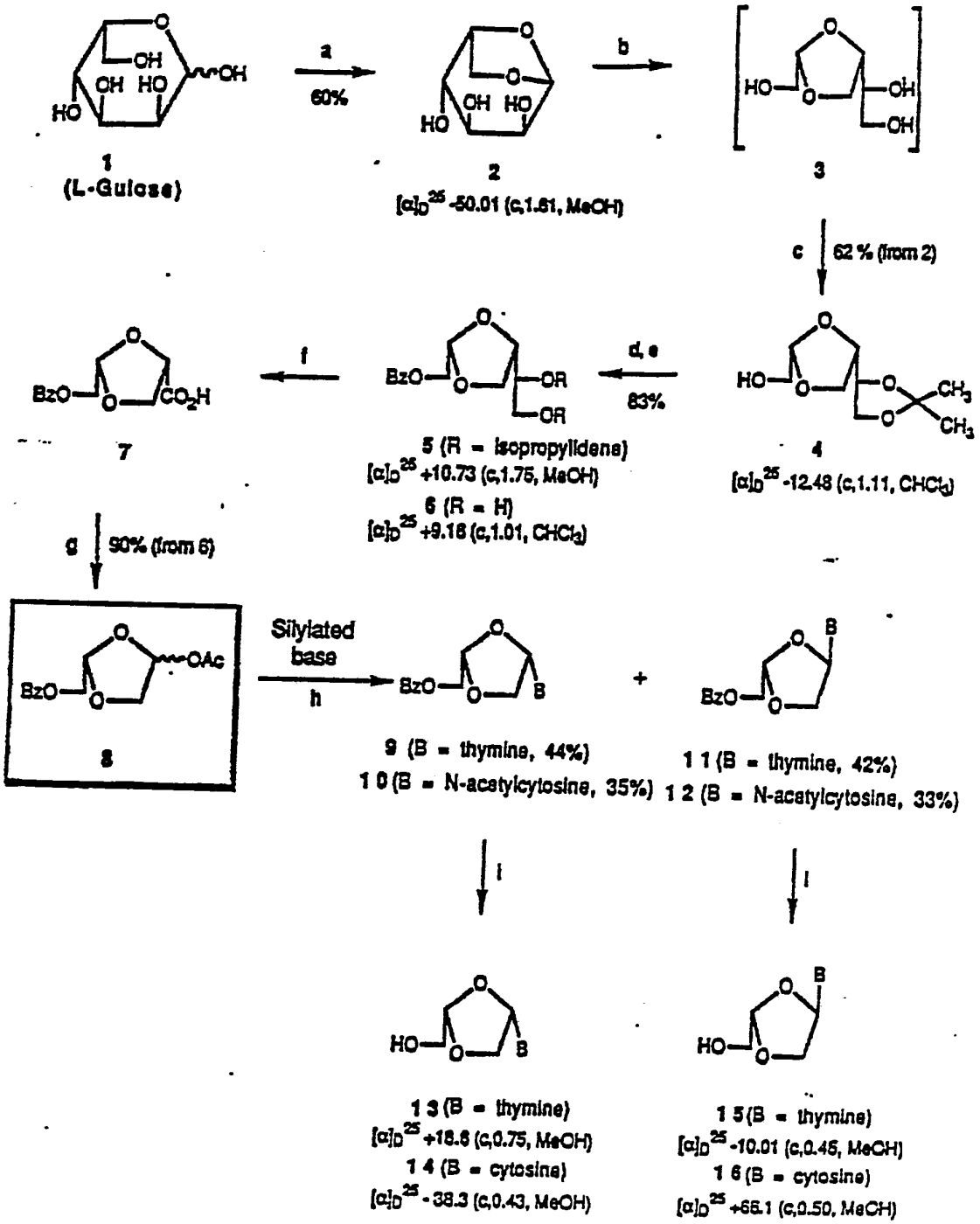
As can be seen the therapeutic index for -OddC should be equal or better than -SddC, in fact, from an economical point of view, -OddC may be even more valuable than -SddC due to its low ID_{50} (anti HBV).

SCHEME 1



a) p-TsOH, $CuSO_4$, Acetone. b) DIBAL, Toluene, -78 °C. c) 0.5 N HCl, reflux. d) I. $NaIO_4$, MeOH, H_2O. II. $NaBH_4$. e) p-TsOH, Acetone.
f) BzCl, Pyr., CH_2Cl_2. g) p-TsOH, MeOH. h) $NaIO_4$, RuO_2, CH_3CN:CCl_4:H_2O (2:2:3). i) $Pb(OAc)_4$, THF.

SCHEME 2

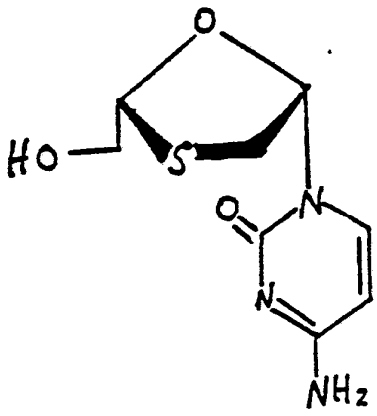


Reagents : a) 0.5N HCl, 100 °C. b) I. $NaIO_4$, MeOH, H_2O, 0 °C. II. $NaBH_4$. c) p-TsOH, Acetone, RT. d) BzCl, Pyr., CH_2Cl_2, RT. e) p-TsOH, MeOH, RT. f) $NaIO_4$, RuO_2, CH_3CN:CCl_4:H_2O (2:2:3), RT. g) $Pb(OAc)_4$, THF, RT. h) TMSOTf, $ClCH_2CH_2Cl$, 0 °C. i) NH_3/MeOH, RT

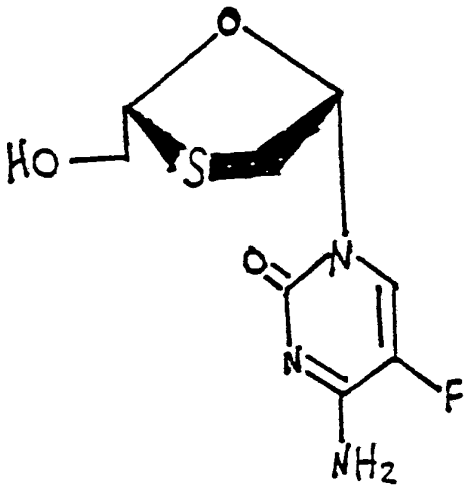
It will be appreciated that the instant specification is set forth by way of illustration, and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

What Is Claimed Is:

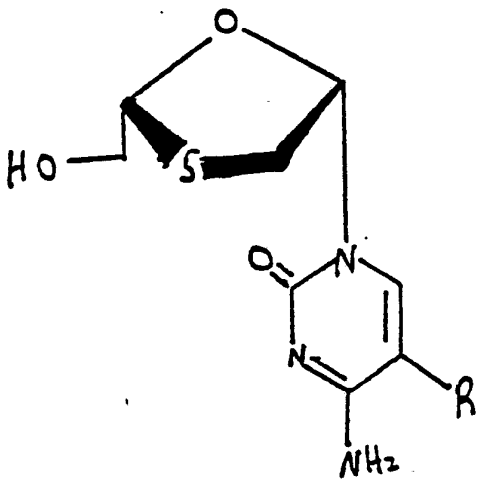
1. (-)3'-Thia-2',3'-dideoxycytidine of the following formula:



2. (-)5-Fluoro-3'-thia-2',3'-dideoxycytidine of the following formula:



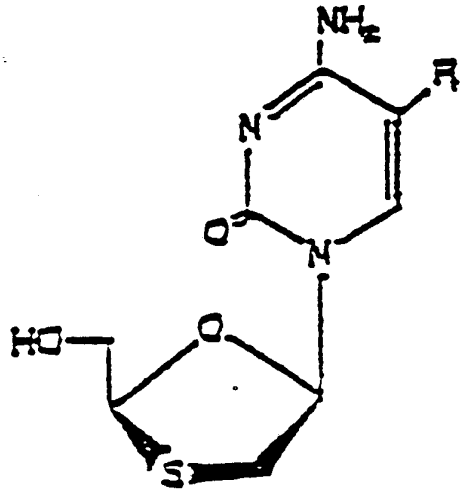
3. A method of treating a patient suffering from hepatitis B virus or preventing hepatitis B virus infection comprising administering to said patient an effective amount of an active compound selected from the group consisting of (a) a compound having the formula (I)



(I)

wherein R is H or F, and (b) ß-dioxolane-cytosine, or a salt of said active compound or an ester of said active compound, either alone or in admixture within a diluent.

4. The method of claim 3, wherein said active compound is the compound of formula (I) and is in admixture with a compound of the following formula (I"):



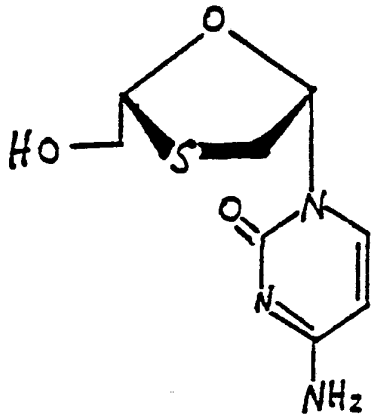
(I")

wherein R is H or F, or a salt or ester thereof either alone or in admixture with a diluent.

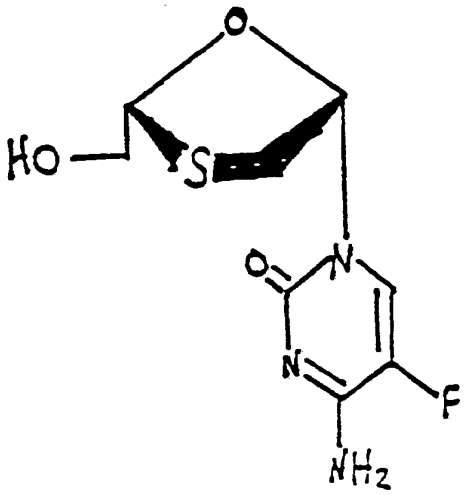
5. The method of claim 4, wherein R is H.

6. The method of claim 4, wherein R is F.

7. The method of claim 3, wherein the active compound is (-)3'-thia-2',3'-dideoxycytidine of the formula



8. The method of claim 3, wherein the active compound is (-)5-fluoro-3'-thia-2',3'-dideoxycytidine of the formula



9. The method of claim 3, wherein the active compound is (-)L-ß-dioxolane-cytosine.

10. The method of claim 3, wherein an ester of said active compound is administered.

11. The method of claim 7, wherein an ester of said active compound is administered.

12. The method of claim 8, wherein an ester of said active compound is administered.

13. The method of claim 3, wherein said active compound is administered in a dose of 1 to 100 mg/kg of body weight per day and said patient is a human.

14. The method of claim 3, wherein said active compound of formula is administered in a dose of 2 to 50 mg/kg of body weight per day and said patient is a human.

15. The method of claim 3, wherein said active compound is administered in a dose of 2 to 10 mg/kg of body weight per day and said patient is a human.

16. The method of claim 7, wherein said active compound is administered in a dose of 1 to 100 mg/kg of body weight per day and said patient is a human.

17. The method of claim 7, wherein said active compound is administered in a dose of 2 to 50 mg/kg of body weight per day and said patient is a human.

18. The method of claim 7, wherein said active compound is administered in a dose of 2 to 10 mg/kg of body weight per day and said patient is a human.

19. The method of claim 8, wherein said active compound is administered in a dose of 1 to 100 mg/kg of body weight per day and said patient is a human.

20. The method of claim 8, wherein said active compound is administered in a dose of 2 to 50 mg/kg of body weight per day and said patient is a human.

21. The method of claim 8, wherein said active compound is administered in a dose of 2 to 10 mg/kg of body weight per day and said patient is a human.

22. The method of claim 4, wherein said compound of formula (I") is administered in a dose of 1 to 100 mg/kg of body weight per day and said patient is a human.

23. The method of claim 4, wherein said compound of formula (I") is administered in a dose of 2 to 50 mg/kg of body weight per day and said patient is a human.

24. The method of claim 4, wherein said compound of formula (I") is administered in a dose of 2 to 10 mg/kg of body weight per day and said patient is a human.

25. The method of claim 3, wherein said active compound is administered in combination with an anti-viral effective amount of adenine arabinoside or interferon α.

26. A method for preparing (-)3'-thia-2',3'-dideoxycytidine according to claim 1 comprising contacting (±)3'-thia-2',3'-dideoxycytidine with deoxycytidine deaminase, subjecting the resultant mixture to column chromatography and separating out said (-)3'-thia-2',3'-dideoxycytidine.

27. The method of claim 24, wherein the column chromatography is HPLC.

28. A method for preparing (-)5-fluoro-3'-thia-2',3'-dideoxycytidine according to claim 2 comprising contacting (±)5-fluoro-3'-thia-2',3'-dideoxycytidine with deoxycytidine deaminase, subjecting the resultant mixture to column chromatography and separating out said (-)5-fluoro-3'-thia-2',3'-dideoxycytidine.

29. The method of claim 26, wherein the column chromatography is HPLC.

30. The method of claim 28, wherein said contacting is carried out at a temperature of 37°C for 16 hours.

31. The method of claim 29, wherein said contacting is carried out at a temperature of 37°C for 16 hours.

32. (±)ß-Dioxolane-cytosine.

33. (-)-L-ß-Dioxolane-cytosine.

34. A compound of the formula

wherein B_z is a benzoyl group and A_c is an acetyl group.

35. A method of producing an L-isomer of a dioxolane nucleoside analog comprising

(a) contacting L-gulose with an acid,

(b) oxidizing and then reducing the product from step (a) without selective protective to form a dioxolane triol having two adjacent hydroxyl groups,

(c) contacting the dioxolane triol from step (b) with protecting groups to selectively protect the two adjacent hydroxyl groups to form an isopropylidene compound,

(d) contacting the isopropylidene compound from step (c) with a benzoylating group and then conducting hydrolysis,

(e) oxidizing the product from step (d),

(f) subjecting the product from step (e) to an oxidative decarboxylation to form an acetate,

(g) contacting the product (f) with a silylated base and

(h) conducting debenzoylation on the product from step (g).

36. The method of claim 35, wherein in step (a), L-glucose is contacted with HCl at a temperature of 100°C; in step (b), the oxidation is carried out with $NaIO_4$ and the reduction is carried out with $NaBH_4$; in step (c) the protecting group is provided by p-toluene sulfonyl; in step (d), the benzoylating group is provided by benzoylchloride and the hydrolysis is carried out with sulfonyl p-toluene; in step (e) the oxidation is carried out with $NaIO_4$ and RuO_2; in step (f), the oxidative decarboxylation is carried out with $Pb(OAc)_4$ and in step (g) the silylated base is trimethylsilyltriflate.

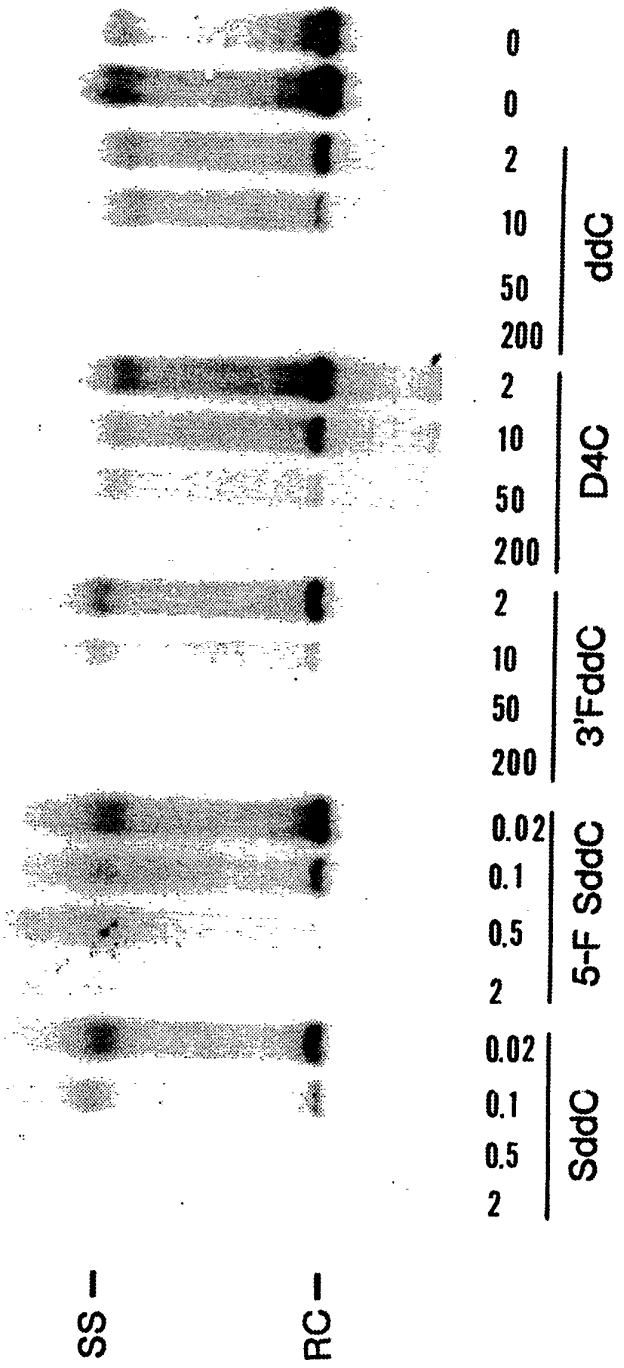

0
0
2
10
50
200
ddC
2
10
50
200
D4C
2
10
50
200
3'FddC
0.02
0.1
0.5
2
5-F SddC
0.02
0.1
0.5
2
SddC
SS
RC

FIG. 1

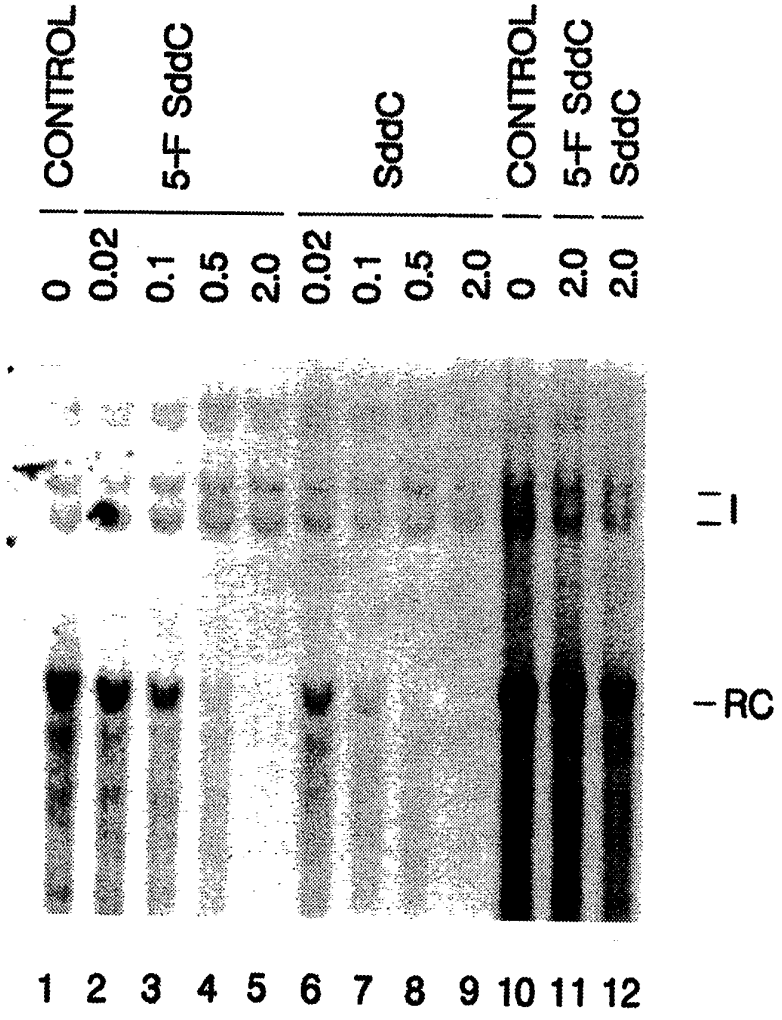

CONTROL
5-F SddC
SddC
CONTROL
5-F SddC
SddC
0
0.02
0.1
0.5
2.0
0.02
0.1
0.5
2.0
0
2.0
2.0
I
RC
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 2

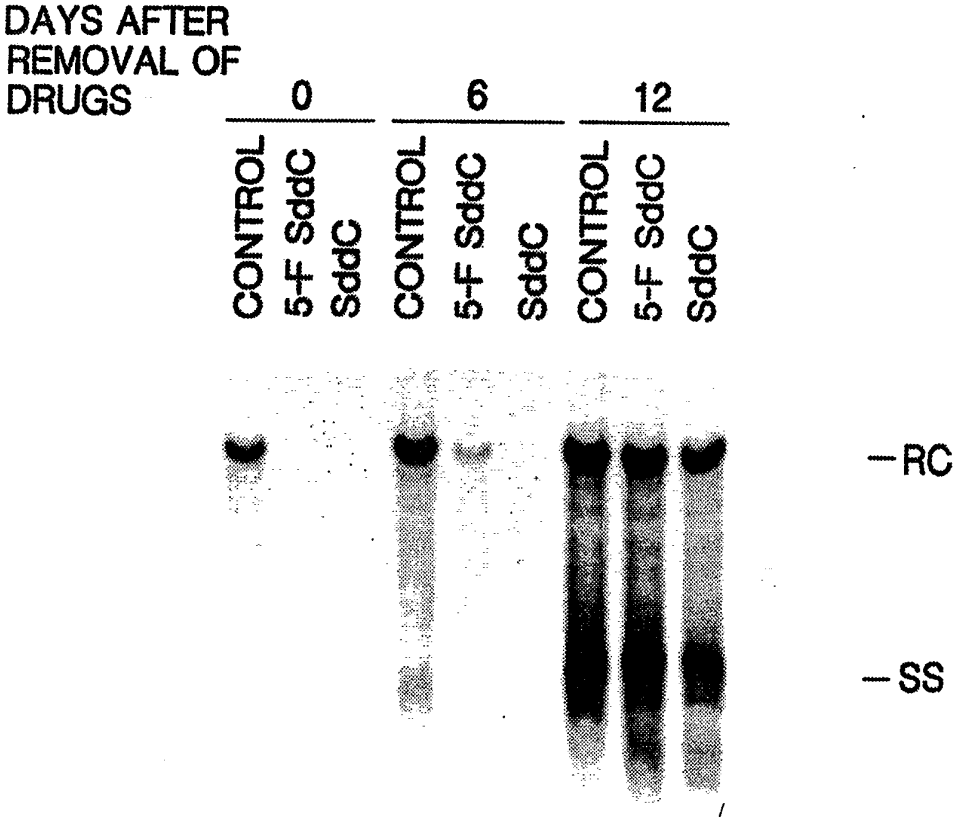

DAYS AFTER REMOVAL OF DRUGS
0
6
12
CONTROL
5-F SddC
SddC
CONTROL
5-F SddC
SddC
CONTROL
5-F SddC
SddC
RC
SS

FIG. 3

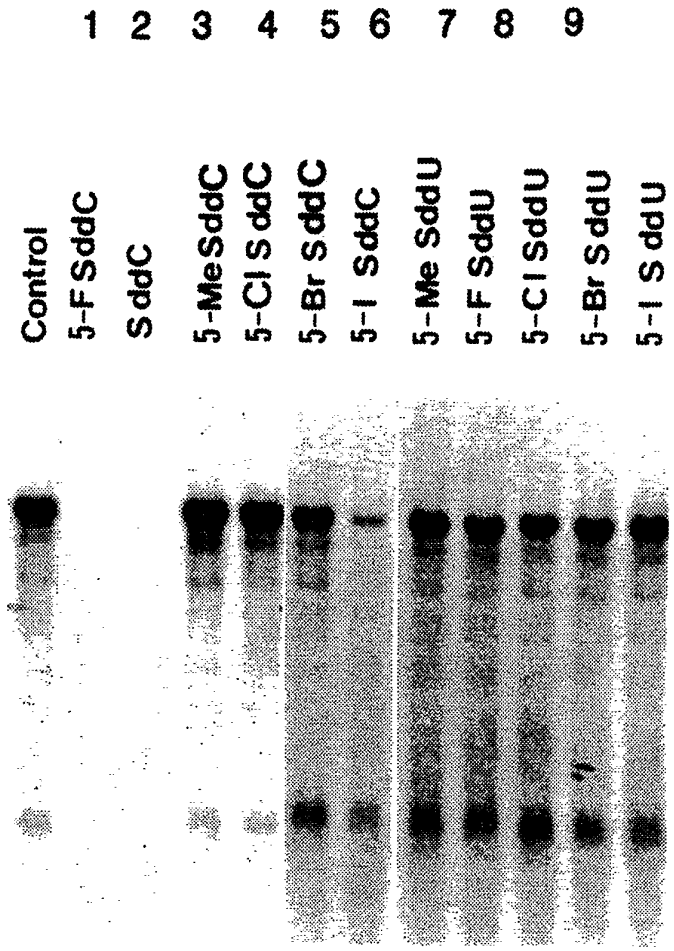

1 2 3 4 5 6 7 8 9
Control
5-F SddC
SddC
5-Me SddC
5-Cl SddC
5-Br SddC
5-I SddC
5-Me SddU
5-F SddU
5-Cl SddU
5-Br SddU
5-I SddU
RC
SS

FIG. 5

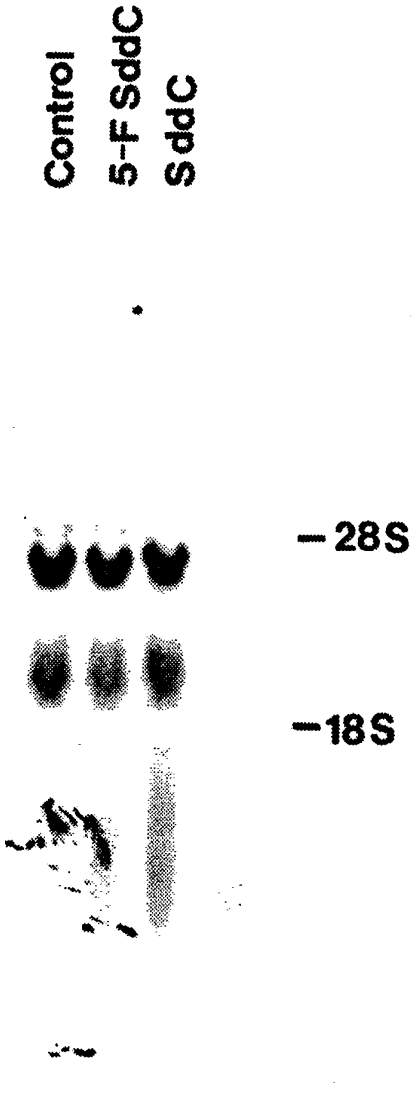


FIG. 4

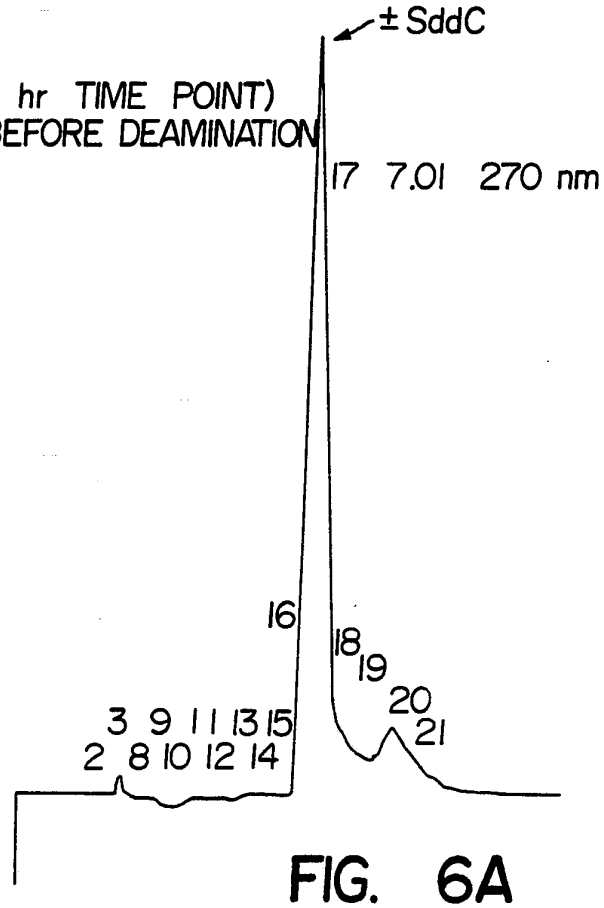

± SddC
(0 hr TIME POINT)
BEFORE DEAMINATION
17 7.01 270 nm
16
18
19
20
21
3 9 11 13 15
2 8 10 12 14

FIG. 6A

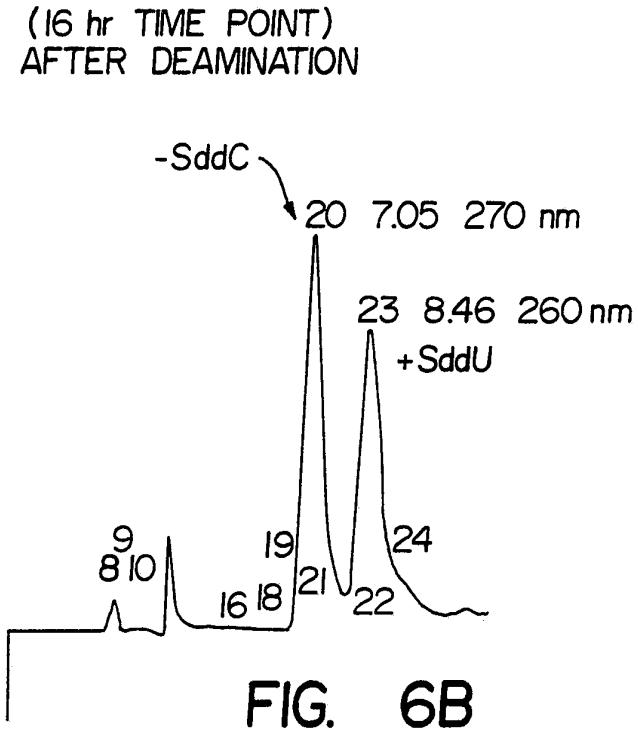

(16 hr TIME POINT)
AFTER DEAMINATION
-SddC
20 7.05 270 nm
23 8.46 260 nm
+SddU
9
8 10
19
24
16 18
21
22

FIG. 6B

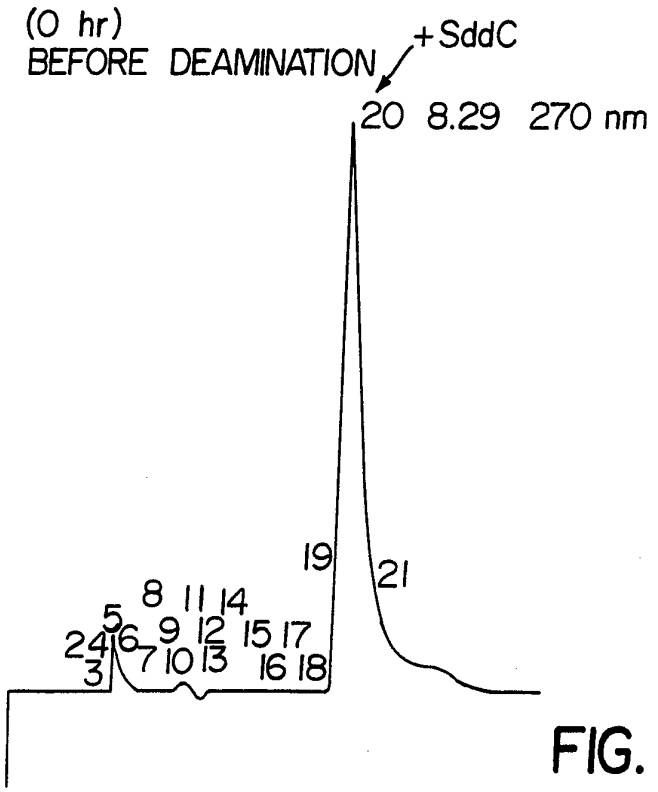

(0 hr)
BEFORE DEAMINATION
+SddC
20 8.29 270 nm
19
21
8 11 14
5
24 6 9 12 15 17
3 7 10 13 16 18

FIG. 7A

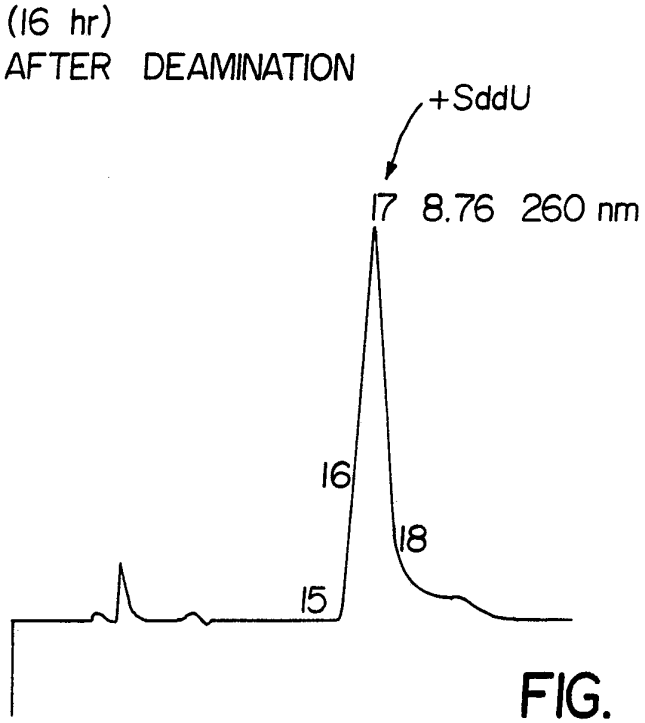

(16 hr)
AFTER DEAMINATION
+SddU
17 8.76 260 nm
16
18
15

FIG. 7B

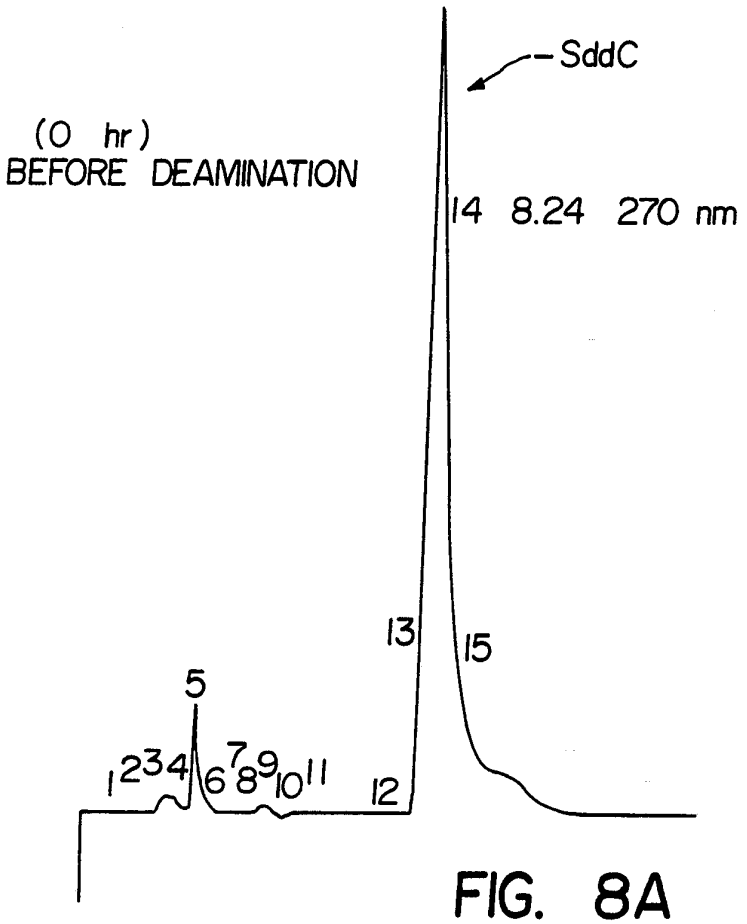

SddC
(0 hr)
BEFORE DEAMINATION
14 8.24 270 nm
13
15
5
1 2 3 4 6 7 8 9 10 11
12

FIG. 8A

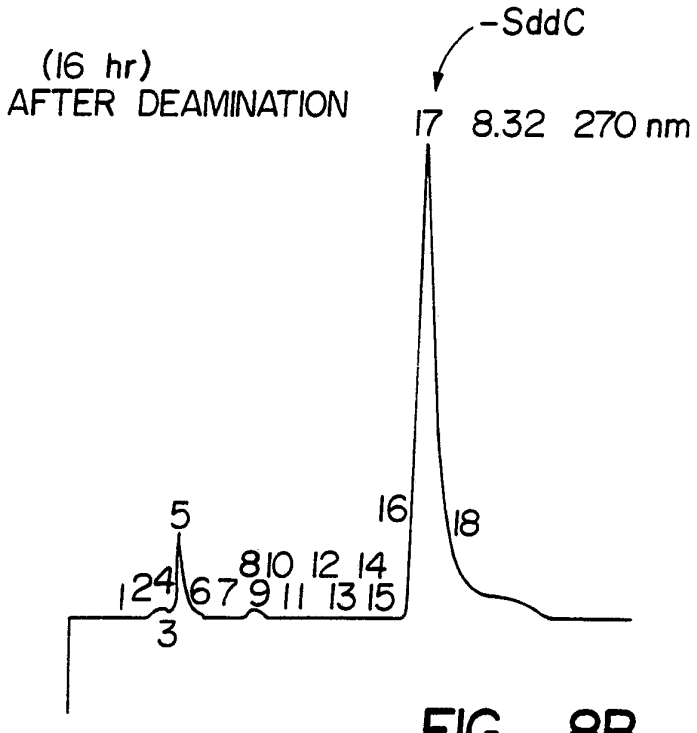

SddC
(16 hr)
AFTER DEAMINATION
17 8.32 270 nm
16
18
5
8 10 12 14
1 2 4 6 7 9 11 13 15
3

FIG. 8B

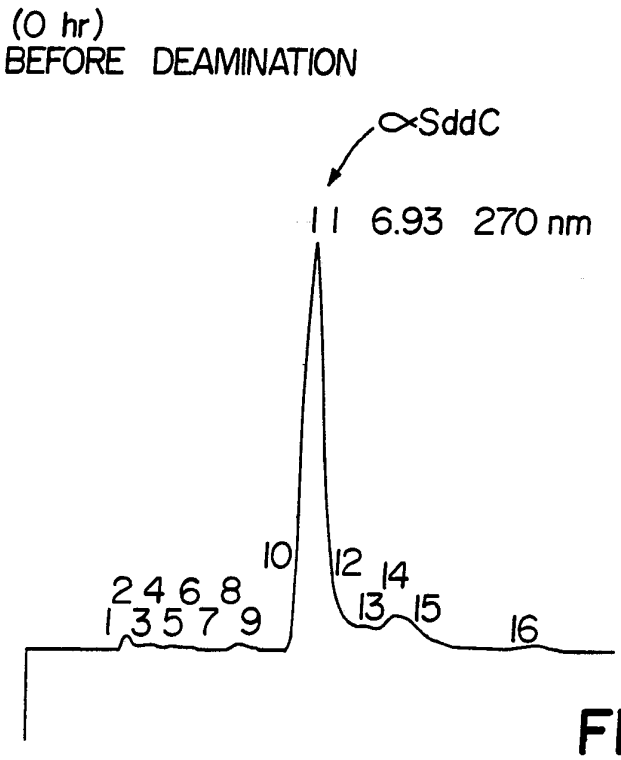

(0 hr)
BEFORE DEAMINATION
∝SddC
11 6.93 270 nm
1 3 5 7 9
2 4 6 8
10
12
13
14
15
16

FIG. 9A

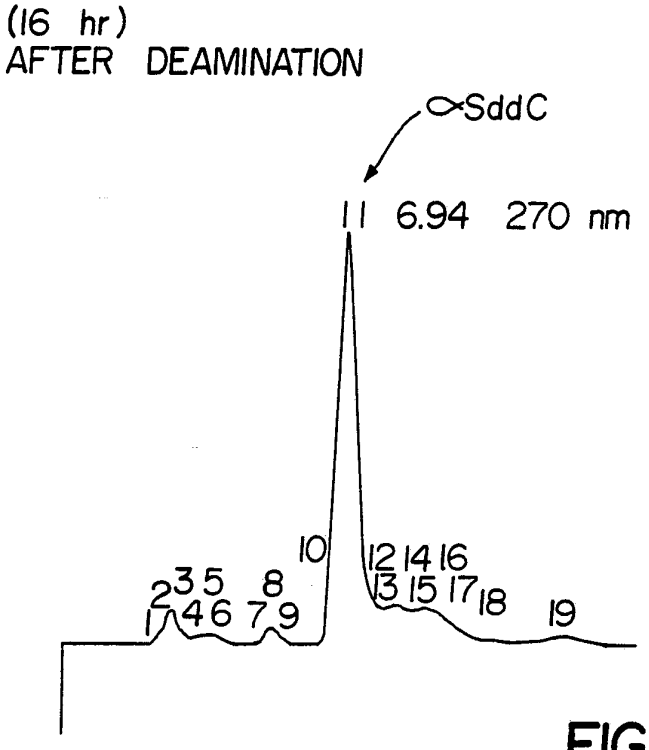

(16 hr)
AFTER DEAMINATION
∝SddC
11 6.94 270 nm
1 2 4 6 7 9
3 5 8
10
12 13
14 15
16 17
18
19

FIG. 9B

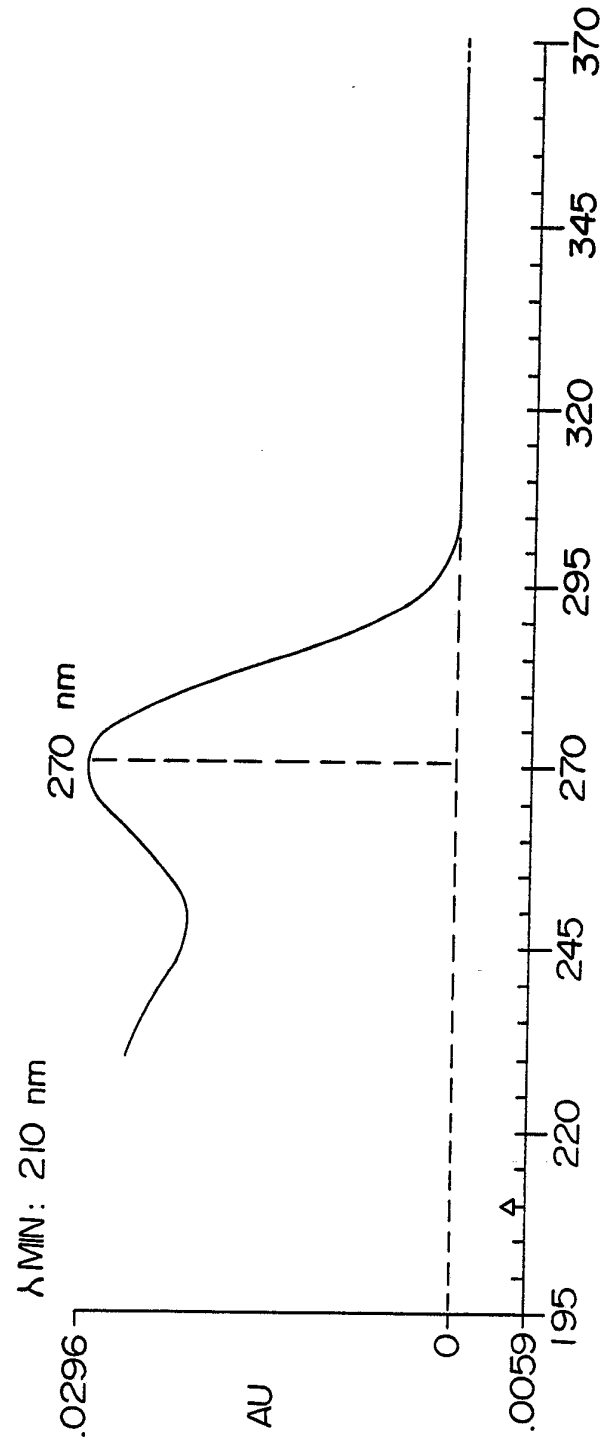


FIG. 10

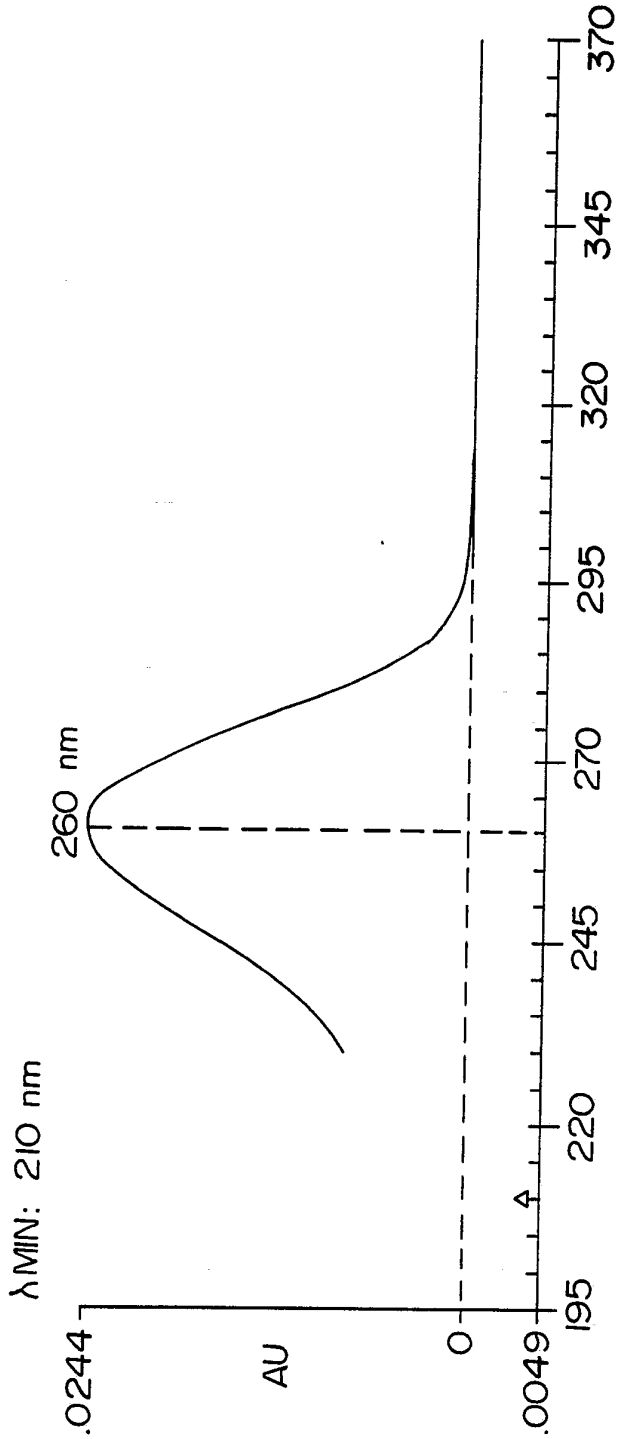


FIG. 11

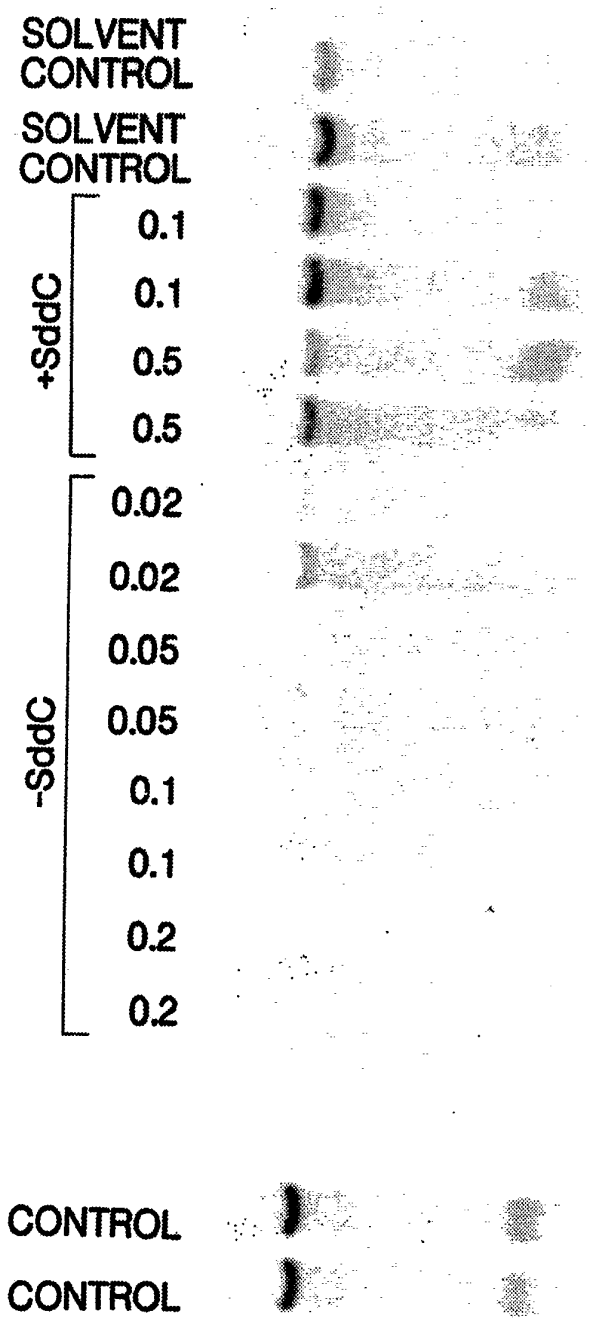

SOLVENT CONTROL
SOLVENT CONTROL
+SddC
0.1
0.1
0.5
0.5
-SddC
0.02
0.02
0.05
0.05
0.1
0.1
0.2
0.2
CONTROL
CONTROL

FIG. 12A

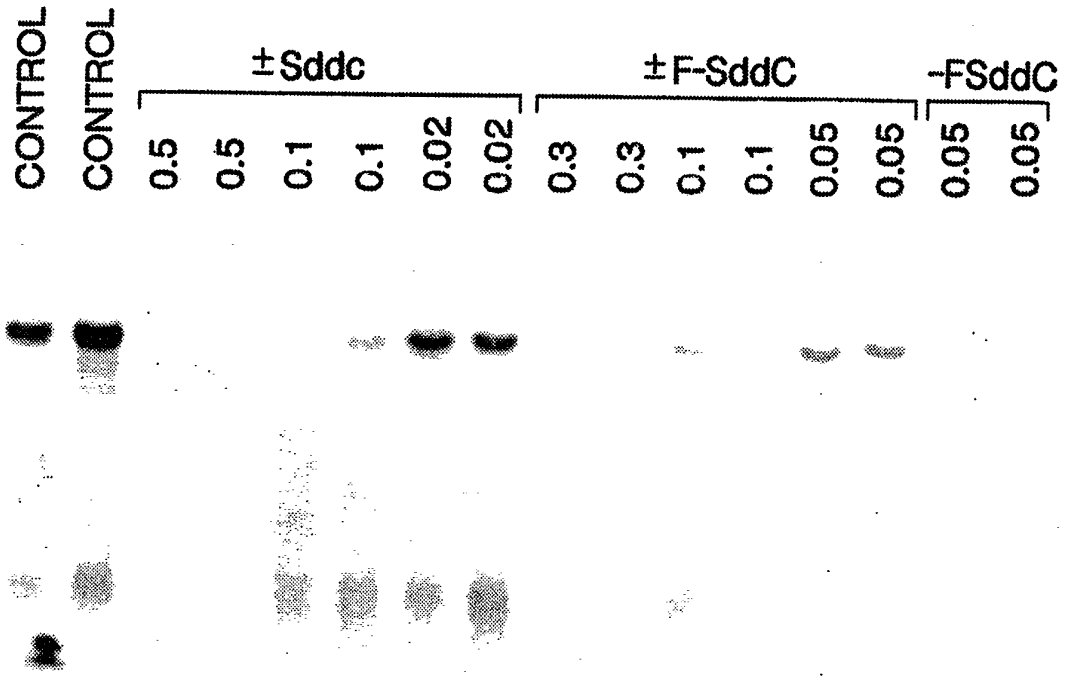

CONTROL
CONTROL
±Sddc
±F-SddC
-FSddC
0.5
0.5
0.1
0.1
0.02
0.02
0.3
0.3
0.1
0.1
0.05
0.05
0.05
0.05

FIG. 12B

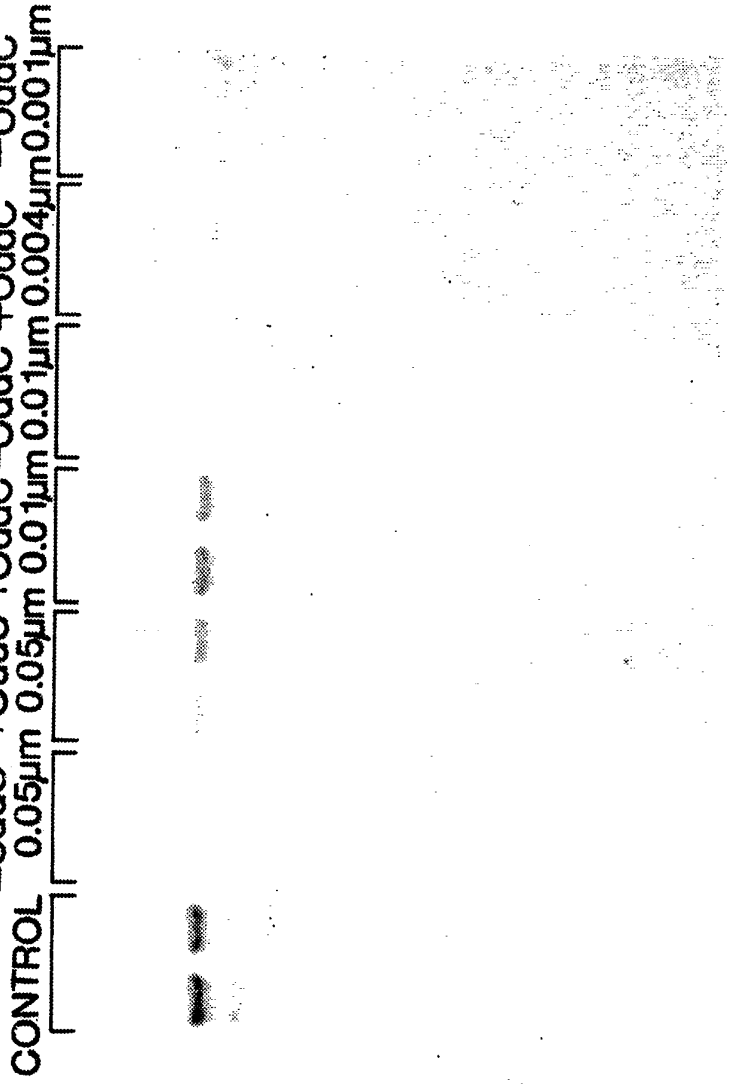

CONTROL
±SddC
+OddC 0.05µm
+OddC 0.01µm
−OddC 0.01µm
+OddC 0.004µm
−OddC 0.001µm

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03144

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 17/00; A61K 31/70
US CL :536/24,125; 514/49
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)

U.S. : 536/24,125; 514/49

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 practicable, 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.
X Y,P	US,A 5,047,407 (BELLEAU ET AL) 10 SEPTEMBER 1991 SEE COLUMN 3, LINE 45-COLUMN 4, LINE 67, COLUMN 5, LINES 28-42.	1-2 3-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 16 SEPTEMBER 1992	Date of mailing of the international search report 24 SEP 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer RAYMOND J. HENLEY III Telephone No. (703) 308-4652

Form PCT/ISA/210 (second sheet)(July 1992)*