Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2188283 C 2003/11/04

(11)(21) 2 188 283

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 1995/04/19
(87) Date publication PCT/PCT Publication Date: 1995/11/02
(45) Date de délivrance/Issue Date: 2003/11/04
(85) Entrée phase nationale/National Entry: 1996/10/18
(86) N° demande PCT/PCT Application No.: CA 1995/000212
(87) N° publication PCT/PCT Publication No.: 1995/029176
(30) Priorité/Priority: 1994/04/20 (08/230,317) US

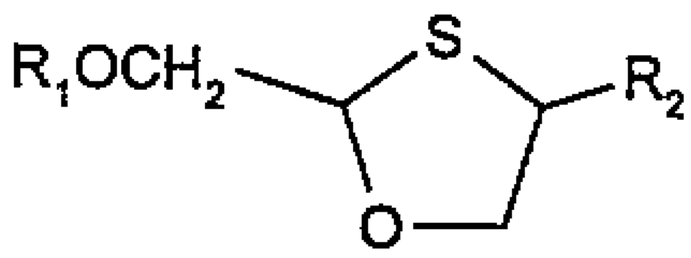
(51) Cl.Int.^{6}/Int.Cl.^{6} C07D 411/04, A61K 31/505

(72) Inventeurs/Inventors:
MANSOUR, TAREK S., CA;
JIN, HAOLUN, CA

(73) Propriétaire/Owner:
BIOCHEM PHARMA INC., CA

(74) Agent: SMART & BIGGAR

(54) Titre : 1,3-OXATHIOLANES SUBSTITUEES A PROPRIETES ANTIVIRALES
(54) Title: SUBSTITUTED 1,3-OXATHIOLANES WITH ANTIVIRAL PROPERTIES



(I)

(57) Abrégé/Abstract:
This invention relates to single enantiomers of novel cis-substituted 1,3-oxathiolanes, of formula (I) wherein R_1 is hydrogen, and R_2 is cytosine or 5-fluorocytosine, and pharmaceutically acceptable salts and esters thereof. This invention also relates to pharmaceutical compositions containing them and to the use of these compounds as antiviral agents, particularly in combination therapy.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ^{6} **:**
C07D 411/04, A61K 31/505

A1

(11) International Publication Number: **WO 95/29176**

(43) International Publication Date: 2 November 1995 (02.11.95)

(21) International Application Number: PCT/CA95/00212

(22) International Filing Date: 19 April 1995 (19.04.95)

(30) Priority Data:
08/230,317 20 April 1994 (20.04.94) US

(71) Applicant *(for all designated States except US)*: BIOCHEM PHARMA INC. [CA/CA]; 275 Armand-Frappier Boulevard, Laval, Quebec H7V 4A7 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MANSOUR, Tarek, S. [CA/CA]; Apartment 102, 3555 University Street, Montreal, Quebec H3A 2B1 (CA). JIN, Haolun [CN/CA]; Apartment 308, 9880 Gouin Boulevard, Montreal, Quebec H8Y 3H3 (CA).

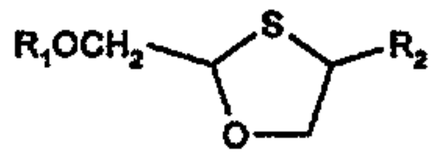
(74) Agent: MBM & CO.; 1000-100 Sparks Street, Ottawa, Ontario K1P 5B7 (CA).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

2188283

(54) Title: SUBSTITUTED 1,3-OXATHIOLANES WITH ANTIVIRAL PROPERTIES



(I)

(57) Abstract

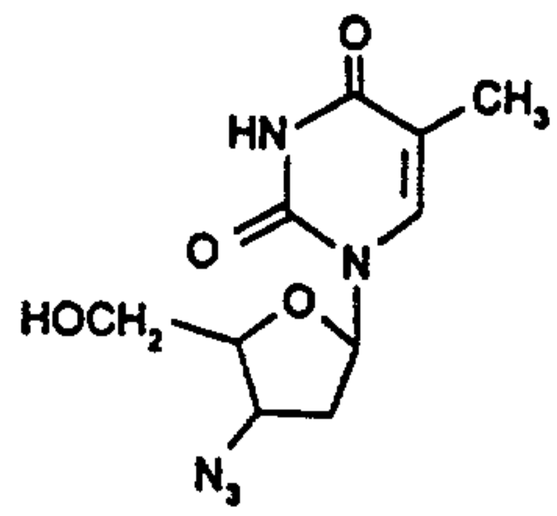
This invention relates to single enantiomers of novel *cis*-substituted 1,3-oxathiolanes, of formula (I) wherein R_1 is hydrogen, and R_2 is cytosine or 5-fluorocytosine, and pharmaceutically acceptable salts and esters thereof. This invention also relates to pharmaceutical compositions containing them and to the use of these compounds as antiviral agents, particularly in combination therapy.

SUBSTITUTED 1,3-OXATHIOLANES WITH ANTIVIRAL PROPERTIES

The present invention relates to novel substituted 1,3-oxathiolane compounds having pharmacological activity, to pharmaceutical compositions containing them, and to the use of these compounds in the antiviral treatment of mammals.

Retroviral infections are a serious cause of disease, most notably, the acquired immunodeficiency syndrome (AIDS). The human immunodeficiency virus (HIV) has been recognized as the etiologic agent of AIDS. Compounds having an inhibitory effect on HIV multiplication or otherwise effective in the therapy of retroviral infections are being actively sought.

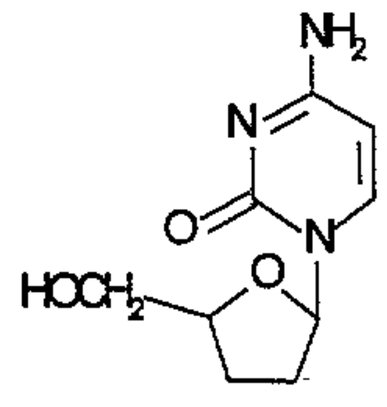
H. Mitsuya et al., "3'-Azido-3'-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro", Proc. Natl. Acad. Sci. USA, 82, pp. 7096-7100 (1985), refers to 3'-azido-3'-deoxythymidine of formula (A), commonly referred to as AZT. This compound is said to be useful in providing some protection for AIDS carriers against the cytopathogenic effect of immunodeficiency virus (HIV).



(A)

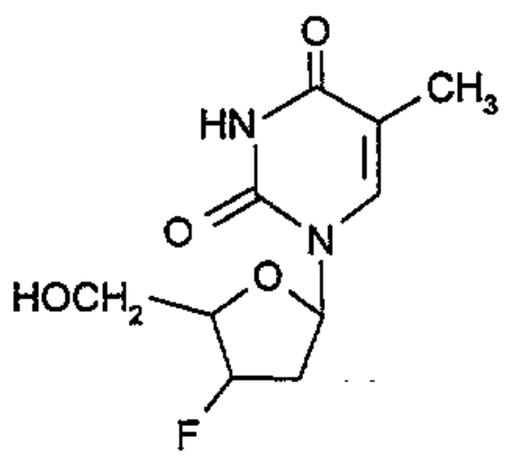
SUBSTITUTE SHEET

H. Mitsuya and S. Broder, "Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides", Proc. Natl. Acad. Sci. USA, 83, pp. 1911-15 (1986), have also referred to a group of 2',3'-dideoxynucleosides shown in formula (B) which are said to possess protective activity against HIV-induced cytopathogenicity.

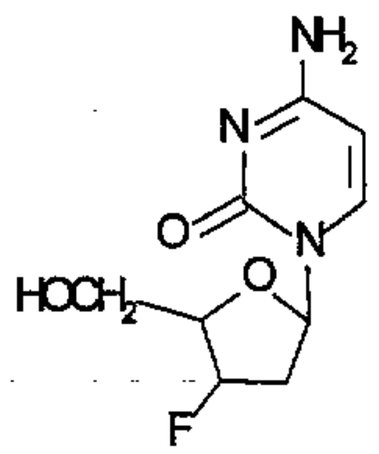


(B)

P. Herdewijn et al., "3'-Substituted 2',3'-dideoxynucleoside analogues as potential anti-HIV(HTLV-III/LAV) agents", J. Med. Chem., 30, pp. 1270-1278 (1987), describe the anti-HIV activity of a series of 3'-substituted nucleoside analogues. While 3'-fluoro analogues of 3'-deoxythymidine and 2',3'-dideoxy-cytidine shown in formulas (C) and (D) are found to possess potent antiretroviral activity, substituents linked to the 3'-carbon via a thio or oxygen bridge did not yield active products.



(C)

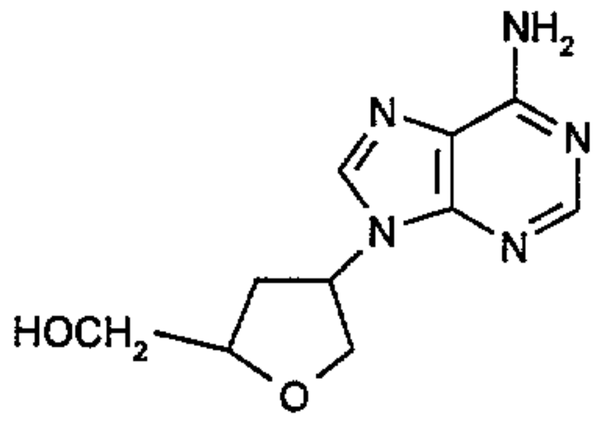


(D)

Analysis of molecular conformation studies in P. Van Roey et al., "Correlation between preferred sugar ring conformation and activity of nucleoside analogues against human immunodeficiency virus", Proc. Natl. Acad. Sci. USA,

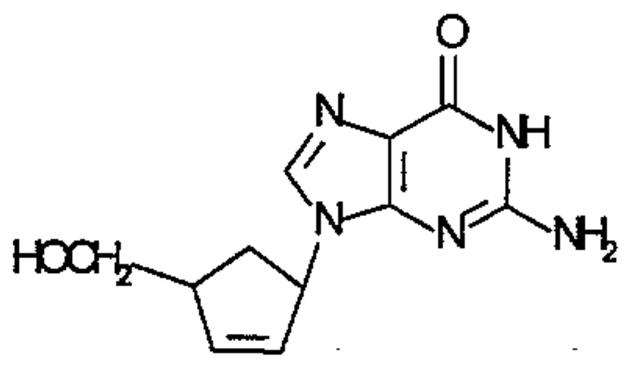
86(10), pp. 3929-3933 (1989), indicate that active anti-HIV nucleoside analogues have 3' carbon conformations on the side opposite to the base.

D. Huryn et al., "Synthesis of iso-ddA, member of a novel class of anti-HIV agents", Tetrahedron Lett., 30(46), pp. 6259-6262 (1989), refer to the iso-nucleoside analogue of formula (E) as a stable inhibitor of HIV replication.

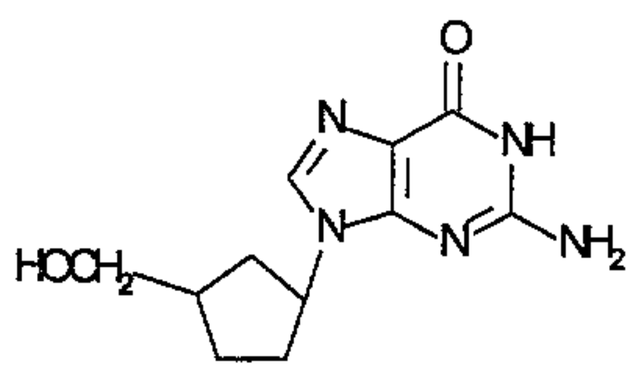


(E)

R. Vince and M. Hua, "Synthesis and anti-HIV activity of carbocyclic 2',3'-didehydro-2',3'-dideoxy 2,6-disubstituted purine nucleosides", J. Med. Chem., 33(1), pp. 17-21 (1990), describe the analogues shown in formulas (F) and (G) as having anti-HIV activity. The unsaturated analogue (F) shows greater selectivity and potency as an inhibitor of HIV replication than the saturated analog (G).

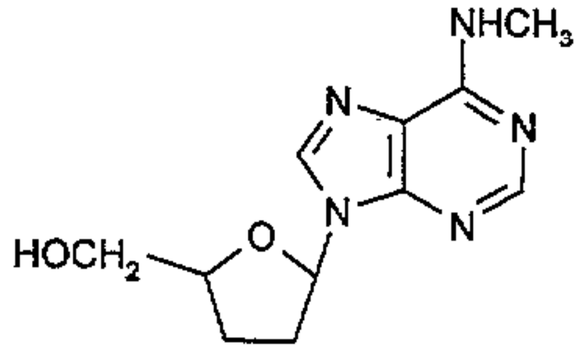


(F)



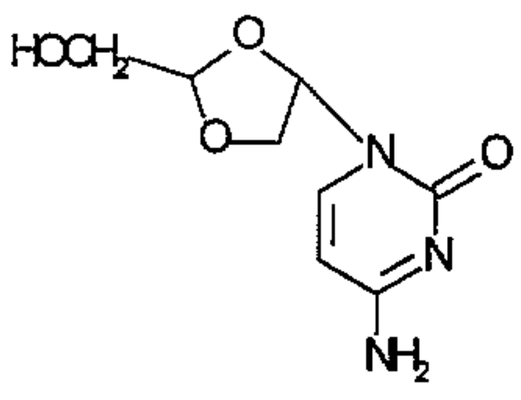
(G)

C. Chu et al., "Synthesis and structure-activity relationships of 6-substituted 2',3'-dideoxypurine nucleosides as potential anti-human immunodeficiency virus agents", J. Med. Chem., 33(6), pp. 1553-1561 (1990), describe the N_6-methyl derivative shown in formula (H) as having greater potency against HIV than unmethylated 2',3'-dideoxyadenosine.

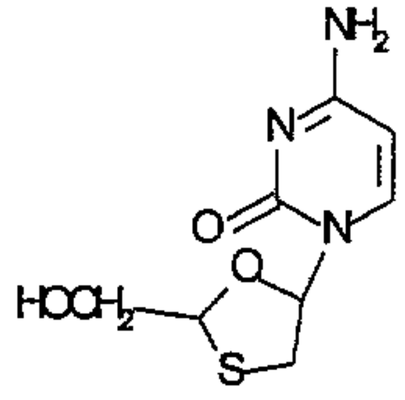


(H)

Finally, B. Belleau et al., "Design and activity of a novel class of nucleoside analogues effective against HIV-1", Abstracts of papers, Fifth International Conference on AIDS, Montreal, T.C.O. 1, p. 515 (1989), refer to dioxolanes and oxathiolanes of formulas (J) and (K) as having potent anti-HIV activity.



(J)



(K)

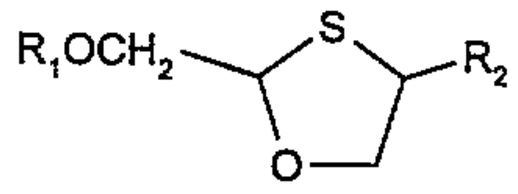
The *cis* isomer of formula (K) has been found to be active against HIV and HBV, and its unnatural enantiomer ((2R,5S *cis*) referred to as "the (-) enantiomer" has been found to have surprisingly low toxicity. Now named lamivudine or "3TC™", this new anti-viral drug is becoming the treatment of choice for combination therapy of AIDS patients and for sole therapy for HBV patients.

Although lamivudine (3TC™) has been found to be an extremely interesting compound in the clinic, there is always the possibility that the patient develops virus strains that are resistant to it after prolonged periods of treatment. There is therefore, still a need to develop anti-viral agents that are active against nucleoside-resistant viral strains, in particular, against 3TC™-resistant viral strains.

SUMMARY OF THE INVENTION

Classes of compounds known as 2-substituted 4-substituted 1,3-oxathiolanes have been found to have potent antiviral activity. In particular, these compounds have been found to act as potent inhibitors of HIV-1 replication in T-lymphocytes over a prolonged period of time with less cytotoxic side effects than compounds known in the art. These compounds have also been found active against 3TC-resistant HIV strains. These compounds are also useful in prophylaxis and treatment of hepatitis B virus infections.

There are accordingly provided in a first aspect of this invention a single enantiomer of compounds of formula (I) in the *cis* configuration:



(I)

wherein R_1 is hydrogen,

R_2 is cytosine or 5-fluorocytosine, and pharmaceutically acceptable salts and esters thereof.

As used herein, "a pharmaceutically acceptable salt, ester, or salt of such ester, of a compound of formula (I) or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) a compound of formula (I) or an antivirally active metabolite or residue thereof.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, p-toluene 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 the invention 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 N-(C_{1-4} alkyl)$_4^+$ salts.

It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable salts and esters thereof, at functional groups in both the cytosine moiety, and at the hydroxymethyl group of the oxathiolane ring. Modification at all such functional groups is included within the scope of the invention. However, of particular interest are pharmaceutically acceptable salts and esters (e.g., esters or esters of amino acids) obtained by modification of the 2-hydroxymethyl group of the oxathiolane ring.

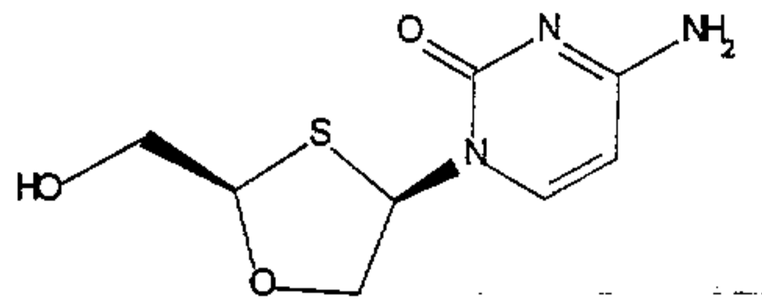
Preferred esters of the compounds of formula (I) include the compounds in which R_1 is replaced by a carboxyl function R-(CO) in which the non-carbonyl moiety R of the ester grouping is selected from hydrogen, straight or branched chain alkyl (e.g., methyl, ethyl, n-propyl, t-butyl, n-butyl), alkoxyalkyl (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 aralkylsulphonyl (e.g., methanesulphonyl); sulfate esters; amino acid esters (e.g., L-valyl or L-isoleucyl) and mono, di- or triphosphate 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, E. Hahn et al., "Nucleotide dimers as anti-human immunodeficiency virus agents", Nucleotide Analogues As Antiviral Agents, J.C. Martin, Ed. Symposium Series #401, American Chemical Society, pp. 156-159 (1989) and M. Busso et al., "Nucleotide dimers suppress HIV expression in vitro", AIDS Research and Human Retroviruses, 4(6), pp. 449-455 (1988).

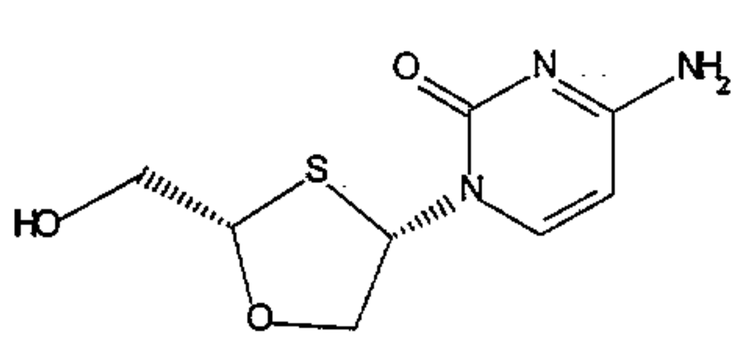
Specific compounds of formula (I) include:

Compound #1 :



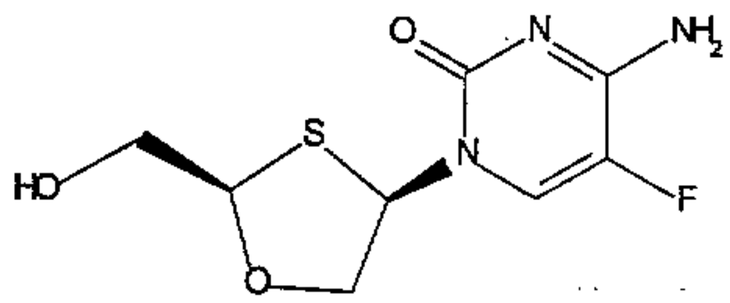
2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane;

compound #2:



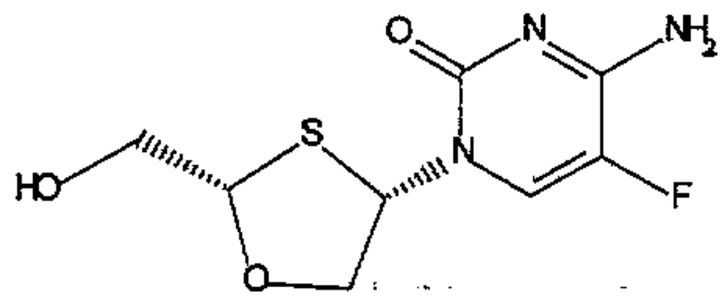
2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane;

compound #3:



2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; and

compound #4



2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane;

and pharmaceutically acceptable salts and esters thereof.

In the processes for preparing the compounds of this invention, the following definitions are used:

R_2 is cytosine or 5-fluorocytosine;

R_W is hydrogen, trisubstituted silyl, C_{1-6} alkyl, aralkyl such as benzyl or trityl, C_{1-16} acyl, preferably a benzoyl or a benzoyl substituted in any position by at least one halogen (bromine, chlorine, fluorine or iodine), C_{1-6} alkyl, C_{1-6} alkoxy, nitro, or trifluoromethyl group;

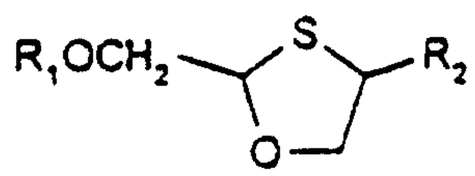
R_X is C_{1-6} alkyl; and

L is a "leaving group", i.e., an atom or group which is displaceable upon reaction with an appropriate base, with or without a Lewis acid. Suitable leaving groups include acyloxy groups, alkoxy groups, e.g., alkoxy carbonyl groups such as ethoxy carbonyl; halogens such as iodine, bromine, chlorine, or fluorine; amido; azido; isocyanato; substituted or unsubstituted, saturated or unsaturated thiolates, such as thiomethyl or thiophenyl; substituted or unsubstituted, saturated or unsaturated seleno or selenino compounds, such as phenyl selenide or alkyl selenide; and substituted or unsubstituted, saturated or unsaturated aliphatic or aromatic ketones such as methyl ketone.

A suitable leaving group may also be -OR, where R is a substituted or unsubstituted, saturated or unsaturated alkyl group, e.g., C_{1-6} alkyl or alkenyl group; 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; a substituted or unsubstituted, saturated or unsaturated alkoxy or

aryloxy carbonyl group, such as methyl carbonate and phenyl carbonate; substituted or unsubstituted sulphonyl imidazolide; substituted or unsubstituted aliphatic or aromatic amino carbonyl group, such as phenyl carbamate; substituted or unsubstituted alkyl imidate group such as trichloroacetamidate; substituted or unsubstituted, saturated or unsaturated phosphonates, such as diethylphosphonate; substituted or unsubstituted aliphatic or aromatic sulphonyl group, such as tosylate; or hydrogen.

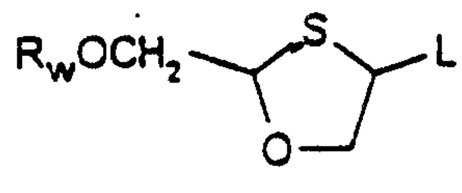
Oxathiolane compounds of formula (I),



(I)

and pharmaceutically acceptable, salts and esters, may be prepared according to the processes discussed herein or by any method known in the art for the preparation of compounds of analogous structure. The compound of this invention can be produced by the methods described by Mansour et al., "Anti-Human Immunodeficiency Virus and Anti-Hepatitis-B Virus Activities and Toxicities of the Enantiomers of 2'-Deoxy-3'-oxa-4'-thiacytidine and Their 5-Fluoro Analogues *in vitro*", J. Med. Chem., 1995, Vol. 38, No. 1, pp. 1-4.

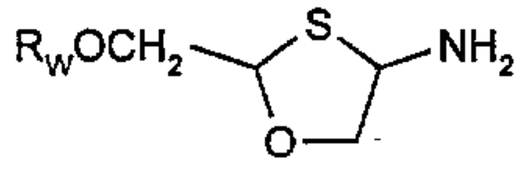
In one such process for producing oxathiolanes of this invention, a compound of formula (V),



(V)

wherein R_W is hydrogen or a hydroxyl protecting group and L is a displaceable atom or group, i.e., a leaving group, is reacted with an appropriate base.

In a second process for producing oxathiolanes of this invention, a compound of formula (VI)



(VI)

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.

The 1,3-oxathiolanes of formula (I) may also be prepared, for example, by reaction of an aldehyde of formula (VII)

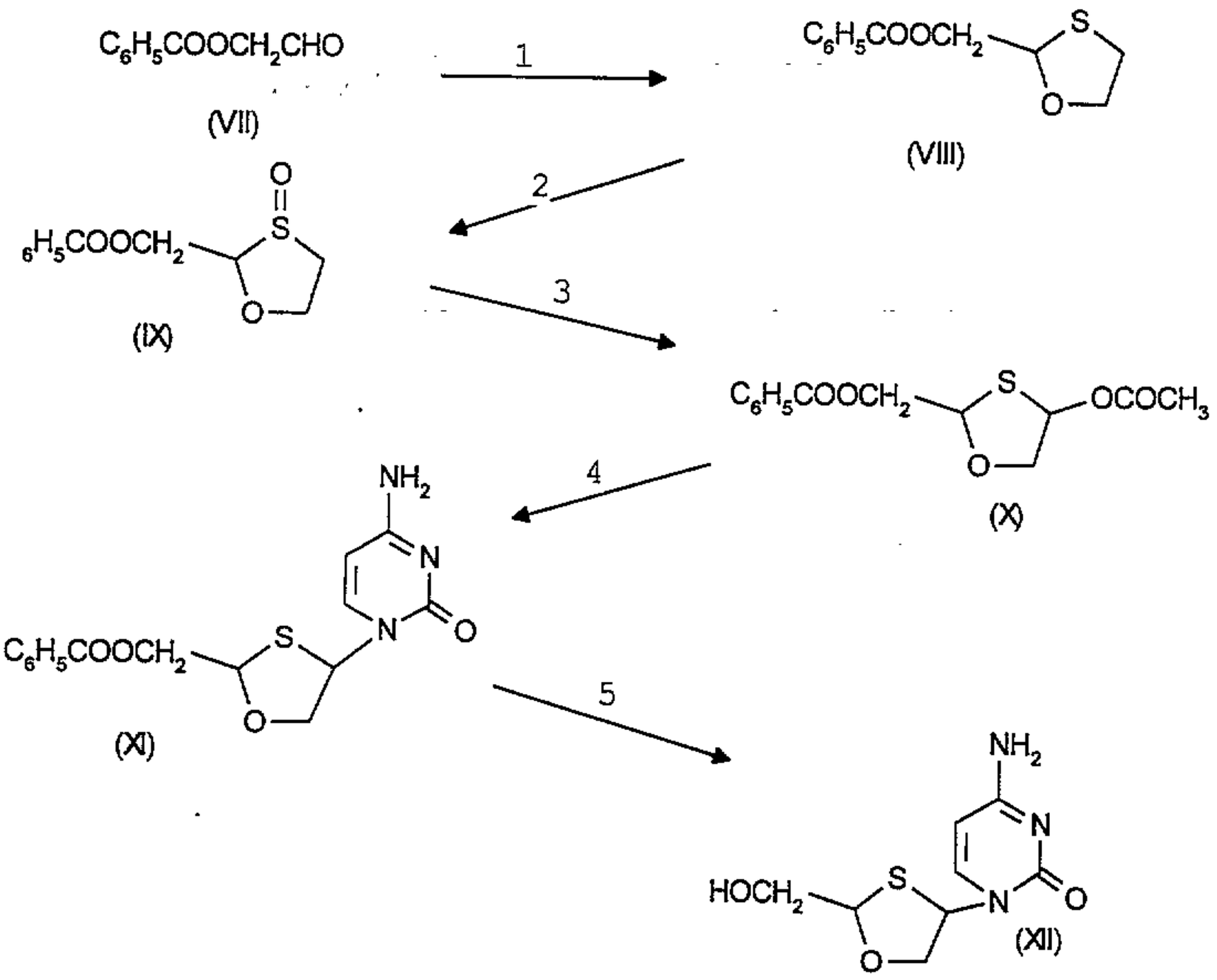
$C_6H_5COOCH_2CHO$ (VII)

with 2-mercaptoethanol in a compatible organic solvent followed by Pummerer rearrangements as is known in the art (T. Durst, "Dimethylsulfoxide in Organic Synthesis", Adv. Org. Chem., E.C. Taylor and B. Wynberg, Eds., 6, pp. 356-365 (1969)) to give 1,3-oxathiolanes of formula (V), which are converted to 1,3-oxathiolanes of formula (I) by methods known in the art of nucleoside chemistry.

Another process for preparing the 1,3-oxathiolanes of formula (I) is illustrated in SCHEME 1.

The various steps involved in the synthesis of 1,3-oxathiolanes of formula (I) as illustrated in SCHEME 1 may be briefly described as follows:

SCHEME 1



Step 1: Benzoyloxyacetaldehyde of formula (VII) or any aldehyde of the formula R_wOCH_2CHO (C.D. Hurd and E.M. Filiachione, "A new approach to the syntheses of aldehyde sugars", J. Am. Chem. Soc., 61, pp. 1156-1159 (1939)) is condensed with a mercaptoalcohol such as 2-mercaptoethanol in a compatible organic solvent, such as toluene, containing a catalytic amount of a strong acid to give the intermediate shown in formula (VIII).

Step 2: The 1,3-oxathiolane of formula (VIII) is then oxidized with a peracid such as magnesium monoperoxyphthalic acid in a compatible organic solvent such as methylene chloride containing a salt such as tetrabutyl ammonium bromide to give the sulfoxide intermediate shown in formula (IX).

74872-53

Step 3: The sulfoxide intermediate shown in formula (IX) is treated with an acid anhydride such as acetic anhydride or any other anhydride of the formula $(R_xCO)_2O$ in the presence of a buffer such as tetra-n-butylammonium acetate to give the 2,4-disubstituted-1,3-oxathiolane of formula (X) (T. Durst, Adv. Org. Chem., 6, pp. 356-365 (1969)).

Step 4: The 1,3-oxathiolane of formula (X) is then reacted with a pyrimidine base or analogue thereof, (e.g., cytosine) previously silylated with, for example, hexamethyldisilazane in a compatible solvent using a Lewis acid or trimethylsilyl triflate to give the intermediate of formula (XI) as *cis* and *trans* isomers. The isomers may be separated, preferably by chromatography, to give pure *cis* (XI) and pure *trans* (XI).

Step 5: The benzoate function of the compound of formula (XI) (*cis* or *trans* isomer), is hydrolyzed using a base such as methanolic ammonia to obtain the compound shown in formula (XII) as *cis*- or *trans*- isomer.

preferably under pressure, to give the product shown in

Many of the reactions in the above-described processes have been extensively reported in the context of pyrimidine nucleoside synthesis, for example, in L.B. Townsend, "Synthesis and reaction of pyrimidine nucleoside", Chemistry of Nucleoside and Nucleotides vol. 1, Eds., Plenum Press, New York (1989) at pages 1-95.

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

The *cis* and *trans* 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.

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.

Where the compound of formula (I) is desired as a single enantiomer it may be obtained either by resolution of the mixture of the two *cis* enantiomers (by chiral HPLC) or by stereospecific synthesis from isometrically pure starting material or any convenient intermediate. Thus, the compound of formula (I) or any convenient intermediate may be obtained by chiral HPLC using a suitable stationary phase for example acetylated β-cyclodextrin or cellulose triacetate and a suitable solvent for example an alcohol such as ethanol or an aqueous solution such as triethyl ammonioum acetate. Alternatively, the compound of formula (I) or any convenient intermediate may be resolved by enzyme mediated enatioselective catabolism with a suitable enzyme such as cytidine deaminase or selective enzymatic degradation of a suitable derivative using a 5'-nucleotidase for example see Storer et al., "The resolution and Absolute Stereochemistry of the Enantiomers of *cis*-1[2(Hydroxomethyl)-1,3-Oxathiolan-5-Yl)Cytosine (BCH-189): Equipotent Anti-HIV Agents", Nucleosides & Nucleotides, 12(2), 225-236 (1993). When the resolution is effected enzymatically, the enzyme may be employed either in solution or in immobilized form. Enzymes in immobilized form are known in the art for example, by adsorption onto a resin such as Eupergit*.

It will be appreciated that the reactions of the above-described processes 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

*Trade-mark

means. Thus, for example, amino groups may be protected by a group selected from aralkyl (e.g., benzyl), acyl or aryl (e.g., 2,4-dinitrophenyl); subsequent removal of the protecting group being effected when desired by hydrolysis or hydrogenolysis 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 benzoyl) 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 triphenylmethyl may similarly be removed by solvolysis, e.g., by hydrolysis under acidic 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.

Pharmaceutically acceptable salts of the compounds of the invention may be prepared as described in United States Patent No. 4,383,114.

Thus, for example, when it is desired to prepare an acid addition salt of a compound of formula (I), the product of any of the above procedures may be converted into 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 basic 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 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.

The compounds of the invention possess anti-viral activity. In particular these compounds are effective in inhibiting the replication of hepatitis B virus and retroviruses, including human retroviruses such as human immunodeficiency viruses (HIV's), the causative agents of AIDS.

There is thus provided as a further aspect of the invention a compound of formula (I) or a pharmaceutically acceptable derivative thereof for use as an active therapeutic agent in particular as an antiviral agent, for example in the treatment of hepatitis B viral and retroviral infections such as HIV infection.

There is also provided in a further or alternative aspect of this invention, use of a compound of formula (I) or a pharmaceutically acceptable derivative

thereof for the manufacture of a medicament for the treatment of a viral infection.

Such viral infections may be, in particular HIV and HBV infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, in particular an infection caused by hepatitis B virus or a retrovirus such as HIV, in a mammal, including man, comprising administration of an effective amount of an antiviral compound of formula (I) or a pharmaceutically acceptable derivative thereof.

The compounds of the invention are also useful in the treatment of AIDS-related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions (such as dementia), anti-HIV antibody-positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpura and opportunistic infections.

The compounds of the invention are also useful in the prevention or progression to clinical illness of individuals who are anti-HIV antibody or HIV-antigen positive and in prophylaxis following exposure to HIV.

The compounds of formula (I) or the pharmaceutically acceptable salts and esters thereof, may also be used for the prevention of viral contamination of biological fluids such as blood or semen *in vitro*.

It will be appreciated by those skilled in the art that references herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of a compound of the invention required for use in treatment 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 at the discretion of the attendant physician or veterinarian. In

general, however, a suitable dose will be in the range from about 1 to about 750 mg/kg of body weight per day, such as 3 to about 120 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

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

The compound is conveniently administered in unit dosage form; for example containing 10 to 1500 mg, conveniently 20 to 1000 mg, most conveniently 50 to 700 mg of active ingredient 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 a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus containing about 0.1 to about 110 mg/kg of the active ingredient. Desirable blood levels may be maintained by a continuous infusion to provide about 0.01 to about 5.0 mg/kg/hour or by intermittent infusions containing about 0.4 to about 15 mg/kg of the active ingredient.

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

The invention thus further provides a pharmaceutical formulation comprising a compound of formula (I) or a pharmaceutically acceptable derivative thereof together with one or more pharmaceutically acceptable carriers thereof 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 thereof.

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 according to the invention may also be formulated for parenteral 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 the invention 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, dispersing agents, suspending agents, thickening agents, or coloring agents.

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

Pharmaceutically 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.

For intra-nasal administration the compounds of the invention 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 also 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 according to the invention 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 according to the invention 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 according to the invention may also contain other active ingredients such as antimicrobial agents, or preservatives.

The compounds of the invention may also be used in combination therapy to avoid the production of resistant viral strains.

In particular, the compounds of the invention may also be used in combination with other therapeutic agents, for example, other anti-infective agents. In particular the compounds of the invention may be employed together with known antiviral agents.

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

Suitable therapeutic agents for use in such combinations include nucleoside analogues such as 3TC™, 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine (ddI), 3'-deoxythymidine, 2',3'-dideoxy-2',3'-didehydro-thymidine, and 2',3'-dideoxy-2',3'-didehydrocytidine and ribavirin; acyclic nucleosides such as acyclovir, ganciclovir, interferons such as alpha-, beta-and gamma-interferon; glucuronation inhibitors such as probenecid; nucleoside transport inhibitors such as dipyridamole; immunomodulators such as interleukin II (IL2) and granulocyte macrophage colony stimulating factor (GM-CSF), erythropoietin, ampligen, thymomodulin, thymopentin, foscarnet, glycosylation inhibitors such as 2-deoxy-D-glucose, castanospermine, 1-deoxynojirimycin; and inhibitors of HIV binding to CD4 receptors such as soluble CD4, CD4 fragments, CD4-hybrid molecules and inhibitors of the HIV aspartyl protease such as L-735,524.

Suitable further therapeutic agents for use in such combinations also include non nucleoside reverse transcriptase inhibitors such as revirapine, TIBO, HEPT, BHAP, MKC-422, α-APA, TSAO, calanolides, and L-697,661.

Preferably, the further therapeutic agent is selected from: 3TC™, AZT, ddC, and ddI.

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

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier thereof comprise a further aspect of the invention.

When the compound of 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 differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

It will be further appreciated that the amount of a compound of the invention and the amount of the further therapeutic agent required for use in treatment will vary not only with the particular compound of the invention and the further therapeutic agent 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 at the discretion of the attendant physician or veterinarian. In general, however, a suitable dose of the compound of the invention will be in the range from about 1 to about 750 mg/kg of body weight per day, such as 3 to about 120 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day. A suitable dose of the further therapeutic agent will be in the range from about 1 to about 750 mg/kg of body weight per day, such as 3 to about 120 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

74872-53

In one aspect, therefore, the present invention provides use of a pharmaceutical formulation for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections in mammals, the pharmaceutical formulation comprising: 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; or a pharmaceutically acceptable salt or ester thereof in an amount effective for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections wherein 3TC™ is 2R-hydroxymethyl-5S-(cytosin-1'-yl)-1,3-oxathiolane and FTC is 2-hydroxymethyl-5-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier.

In another aspect the invention provides a pharmaceutical formulation for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections in mammals, the pharmaceutical formulation comprising: 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; or a pharmaceutically acceptable salt or ester thereof in an amount effective for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier.

74872-53

In a further aspect the invention provides use of: 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; or a pharmaceutically acceptable salt or ester thereof in the preparation of a pharmaceutical formulation for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier.

In yet a further aspect the invention provides a commercial package containing: 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers; or a pharmaceutically acceptable salt or ester thereof, together with instructions for its use for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections.

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

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

EXAMPLES

Example 1

BENZOYLOXYACETALDEHYDE

$C_6H_5COOCH_2CHO$ (VII)

This known intermediate was prepared by portionwise addition of $NaIO_4$ (80 g) to a mixture of 1-benzoyl glycerol (50 g), CH_2Cl_2 (500 ml), and H_2O (25 ml) under vigorous stirring at room temperature. The resulting solution was stirred for 2 hours, $MgSO_4$ (100 g) was added and the solution stirred for an additional 30 minutes. The mixture was filtered, the filtrate evaporated *in vacuo* and the residue distilled *in vacuo* to yield 26 g of pure product.

b.p. 92-94°/0.25 mm

^1H NMR (200 MH_z; TMS as internal reference):

δ(ppm in $CDCl_3$)

9.71 (s, 1H; -C**H**O)

8.11 (d, 2H; aromatic)

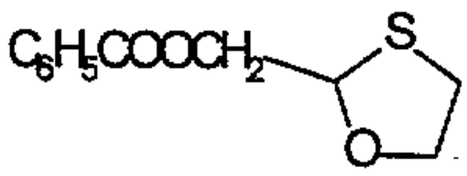
7.60 (m, 1H; aromatic)

7.46 (m, 2H; aromatic)

4.88 (s, 2H; -CH_2CHO)

Example 2

2-BENZOYLOXYMETHYL-1,3-OXATHIOLANE



(VIII)

A mixture of benzoyloxyacetaldehyde (example 1) (6.21 g), 2-mercaptoethanol (3 ml) and para-toluene sulfonic acid (0.2 g) in toluene (150 ml) was heated for 3 hours at refluxing under water removal conditions using a Dean Stark apparatus. The mixture was cooled to room temperature, washed first with aqueous $NaHCO_3$-solution (1 x 50 ml), and then with water (2.5 ml) and dried over $MgSO_4$. The solution was filtered and the filtrate evaporated under reduced pressure. The residue was purified on silica gel using hexane:ethyl acetate (9:1) as eluant. It yielded 7.63 g (90%) of pure product, which was identified by ^1H- and ^{13}C-NMR.

R_f: 0.39 (hexane:ethyl acetate)

^1H-NMR: δ (ppm in $CDCl_3$)

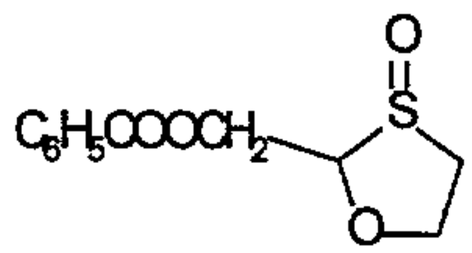
8.03 (m, 2H, aromatic)
7.53 (m, 1H, aromatic)
7.39 (m, 2H, aromatic)
5.41 (dd, 1H, C_2-H)
4.43 (m, 2H, C_2-$CH_2OCC_6H_5$)
4.21 (m, 1H, C_5-H)
3.96 (m, 1H, C_5-H)
2.98 (m, 2H, C_4-H)

^{13}C-NMR: δ (ppm in $CDCl_3$)

166.82, 133.74, 130.35, 128.97, 83.58, 71.87, 66.62 and 32.74

Example 3

2-BENZOYLOXYMETHYL-3-OXO-1,3-OXATHIOLANE



(IX)

Monoperoxyphthalic acid, magnesium salt (MMPP, 28 g) was added portionwise under vigorous stirring to a mixture of 2-benzoyloxymethyl-1,3-oxathiolane (example 2) (20 g), tetrabutyl ammonium bromide (0.4 g) in methylene chloride (200 ml), and water (200 ml). The mixture was stirred at

room temperature for 30 minutes and the organic layer was collected. The aqueous phase was extracted with methylene chloride (3 x 75 ml) and the combined organic layer was washed first with water (2 x 100 ml), then with brine solution (100 ml), dried over $MgSO_4$, and filtered. The filtrate was evaporated *in vacuo* and the residue was purified by chromatography on silica gel using ethyl acetate as eluant to give 18.5 g (86%) of pure product as a mixture of *cis*- and *trans*- isomers in a ratio of 1:2 respectively.

m.p.: 70-72°

^{1}H-NMR: δ (ppm in $CDCl_3$)

8.05 (m, 2H, aromatic, *cis*-isomer)

7.95 (m, 2H, aromatic, *trans*-isomer)

7.56 (m, aromatic)

7.23 (m, aromatic)

4.77 (m, 4H, C_2-H, C_5-H, and C_2-$CH_2OOCC_6H_5$)

4.43 (m, 1H, C_5-H, *trans*-isomer)

4.09 (m, 1H, C_5-H, *cis*-isomer)

3.11 (m, 2H, C_4-H, *trans*-isomer)

2.75 (m, 2H, C_4-H, *cis*-isomer)

^{13}C-NMR: δ (ppm in $CDCl_3$)

cis-isomer:

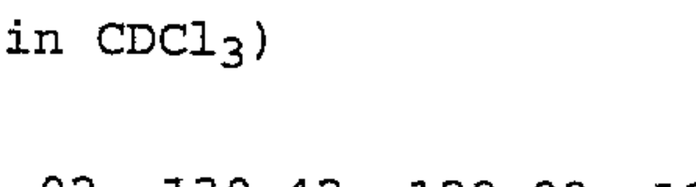
166.64, 134.02, 130.42, 129.88, 129.06, 96.16, 68.83, 59.47 and 54.30

trans-isomer:

166.36, 134.12, 130.29, 129.68, 129.15, 108.07, 70.09, 61.83 and 53.47

Example 4

2-BENZOYLOXYMETHYL-4-ACETOXY-1,3-OXATHIOLANE



(X)

A mixture of 2-benzoyloxymethyl-3-oxo-1,3-oxathiolane (example 3) (10.5 g), tetra-n-butylammonium acetate (17 g) in acetic anhydride (250 ml) was heated at 110 to 120°C under argon for 14 hours and cooled to room temperature. Excess acetic anhydride was removed under reduced pressure. The residue was dissolved in methylene chloride (500 ml), washed first with saturated aqueous $NaHCO_3$ (2 x 200 ml), then with brine solution (200 ml), dried over $MgSO_4$, filtered and evaporated *in vacuo*. The residue was purified by chromatography on silica gel using hexane:ethyl acetate (8:1) as eluant to give 7.4 g (60% yield) of the desired product as a mixture of *cis*-and *trans*- isomers. A small quantity of each isomer was also isolated and characterized by ^1H- and ^{13}C-NMR.

cis-isomer:

R_f: 0.43 (hexane:EtOAc)

^1H-NMR: δ (ppm in $CDCl_3$)

8.05 (m, 2H, aromatic)
7.58 (m, 1H, aromatic)
7.45 (m, 2H, aromatic)
6.24 (d, 1H, C_4-H)
5.50 (t, 1H, C_2-H)
4.61 (d, 1H, C_2-$CH_2OOCC_6H_5$)
4.53 (d, 2H, C_5-H)
3.94 (dd, 1H, C_5-H)
2.05 (s, 3H, CH_3)

trans-isomer:

R_f: 0.43 (hexane:EtOAc 7:3)

^1H-NMR: δ (ppm in $CDCl_3$)

8.04 (m, 2H, aromatic)
7.58 (m, 1H, aromatic)
7.45 (m, 2H, aromatic)
6.27 (dd, 1H, C_4-H)
5.73 (dd, 1H, C_2-H)
4.53 (dd, 1H, C_2-$CH_2OOCC_6H_5$)
4.34 (dd, 1H, C_5-H)

4.26 (dd, 1H, C_2-$CH_2OCC_6H_5$)

4.20 (dd, 1H, C_5-H)

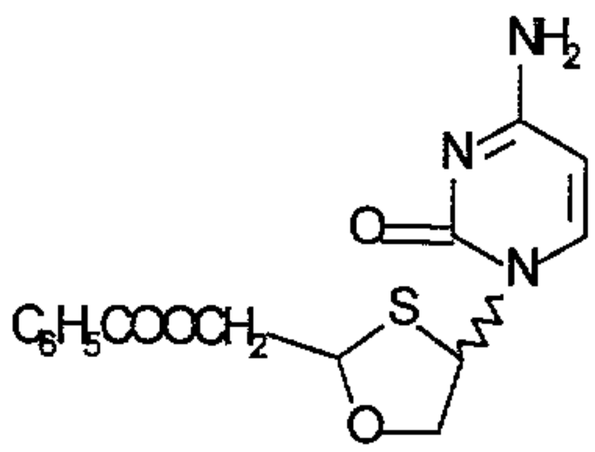
2.09 (s, 3H, CH_3)

^{13}C-NMR: δ (ppm in $CDCl_3$)

177.66, 166.37, 133.46, 129.93, 128.60, 83.76, 81.22, 74.33, 64.65 and 20.79

Example 5

***CIS*- AND *TRANS*-2-BENZOYLOXYMETHYL-4-(CYTOSIN-1'-YL)-1,3-OXATHIOLANE**



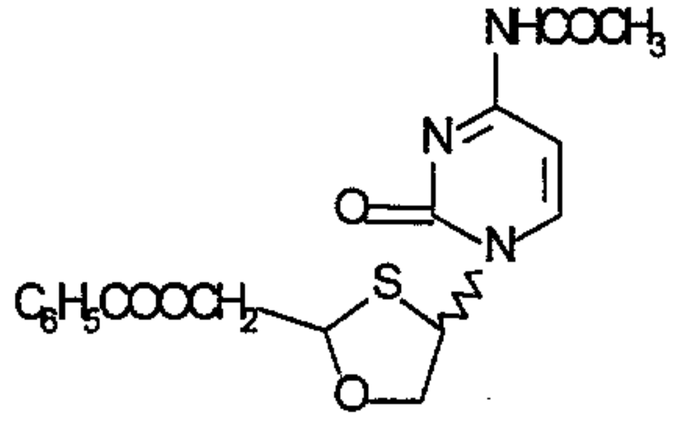
(XXXI)

A mixture of cytosine (206 mg), ammonium sulfate (10 mg) and hexamethyldisilazane (HMDS, 10 ml) was heated at refluxing under argon until a clear solution resulted. Excess reagents were evaporated *in vacuo* and the remaining volatile removed under high vacuum (15 minutes). The solid residue was dissolved in dry methylene chloride (20 ml) and a solution of 2-benzoyloxymethyl-4-acetoxy-1,3-oxathiolane (example 4) (350 mg) in dry methylene chloride (20 ml) was added under argon, followed by a solution of tin IV chloride ($SnCl_4$, 124 ml) in methylene chloride (20 ml) at 0°C. The reaction mixture was stirred under argon overnight at room temperature, then heated at refluxing for 3 hours and cooled to room temperature. The mixture was diluted with methylene chloride (100 ml) and poured while stirring into saturated aqueous $NaHCO_3$. The organic layer was separated by filtration over celite, washed first with water (2 x 75 ml), then with brine solution (100 ml), dried over $MgSO_4$ and filtered. The residue was purified by chromatography on silica gel using ethyl acetate:CH_3OH as the eluant to give 140 mg (35%) of the

desired product as a mixture of *cis*- and *trans*- isomers in a 1:1 ratio as determined by ^{1}H-NMR. These isomers were separated as the N-acetyl derivatives in the next example.

Example 6

CIS- AND TRANS-2-BENZOYLOXYMETHYL-4-(N4'-ACETYL-CYTOSIN-1'-YL)-1,3-OXATHIOLANE



(XXXII)

A solution of the *cis*- and *trans*- mixture of 2-benzoyloxymethyl-4-(cytosin-1'-yl)-1,3-oxathiolane (example 5) (135 mg), 4-dimethylaminopyridine (DMAP, 15 mg) and acetic anhydride (44 ml) in dry pyridine (10 ml) was stirred overnight at room temperature (16 hours) and poured into cold water (100 ml) followed, by extraction with methylene chloride (3 x 50 ml). The extract was washed with water, dried over $MgSO_4$, filtered and evaporated *in vacuo*. Toluene was added to the residue, then evaporated *in vacuo*. Toluene was added to the residue, then evaporated *in vacuo* and the residual oil was purified by chromatography on silica gel using ethyl acetate as eluant to yield 65 mg of pure *trans*-isomer as the fast moving product and 60 mg of pure *cis*-isomer as the low moving product. These were characterized by ^{1}H and ^{13}C-NMR.

cis-isomer:

^{1}H-NMR: δ (ppm in $CDCl_3$)

9.61 (b, 1H, C_4'-$NHCOCH_3$)
8.29 (d, 1H, C_6'-H)
8.06 (m, 2H, aromatic)
7.65 (m, 1H, aromatic)
7.51 (m, 2H, aromatic)
7.25 (d, 1H, C_5'-H)

6.61 (d, 1H, C_4-H)
5.50 (t, 1H, C_2-H)
4.80 (m, 2H, C_2-$CH_2OOCC_6H_5$)
4.48 (d, 1H, C_5-H)
4.05 (dd, 1H, C_5-H)
2.25 (s, 3H, CH_3)

^{13}C-NMR: δ (ppm in $CDCl_3$)
170.93, 166.28, 162.80, 155.76, 146.06, 133.91, 129.90, 128.84, 97.45, 85.88, 78.25, 64.60, 63.53 and 24.71.

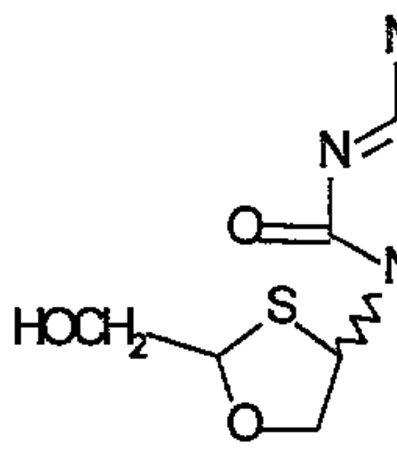
trans-isomer:

^{1}H-NMR: δ (ppm in DMSO d_6)
10.88 (s, 1H, C_4'-$NHCOCH_3$)
8.13 (d, 1H, C_6'-H)
7.96 (m, 2H, aromatic)
7.68 (m, 1H, aromatic)
7.52 (m, 2H, aromatic)
7.20 (d, 1H, C_5'-H)
6.35 (d, 1H, C_4-H)
5.96 (dd, 1H, C_2-H)
4.58 (dd, 1H, C_2-$CH_2OOCC_6H_5$)
4.44 (d, 1H, C_5-H)
4.29 (m, 2H, C_5-H and $CH_2OOCC_6H_5$)
2.07 (s, 3H, CH_3)

^{13}C-NMR: δ (ppm in DMSO d_6)
171.53, 165.84, 162.76, 155.21, 146.59, 134.00, 129.64, 129.23, 96.54, 83.78, 74.24, 64.58, 64.01 and 24.35

Example 7

CIS- AND *TRANS*-2-HYDROXYMETHYL-4-(CYTOSIN-1'-YL)-1,3-OXATHIOLANE



(XXXIII)

cis-isomer **(BCH-270)**:

A solution of *cis*-2-benzoyloxymethyl-4(N4'-acetyl-cytosin-1'-yl)-1,3-oxathiolane (example 6) (54 mg) in methanolic ammonia (50 ml) was stirred overnight at room temperature (16 hours). The solvent was evaporated *in vacuo* and the residue treated with ether yielding 37 mg (90%) of desired product. The product was then characterized by ^{1}H- and ^{13}C-NMR.

m.p.: 213-215°C

UV:(CH_3OH) Lamda max: 270 nm

^{1}H-NMR: δ (ppm in, DMSO d_6)

7.85 (d, 1H, C_6'-H)

7.16 (d, 2H, C_4'-NH_2)

6.34 (d, 1H, C_4-H)

5.76 (d, 1H, C_5'-H)

5.31 (t, 1H, C_2-CH_2OH)

5.18 (t, 1H, C_2-H)

4.40 (d, 1H, C_5-H)

3.92 (dd, 1H, C_5-H)

3.78 (m, 2H, C_2-CH_2OH)

^{13}C-NMR: δ (ppm in DMSO d_6)

165.95, 155.74, 142.39, 94.98, 88.85, 77.29, 62.91 and 62.48

trans-isomer:

A solution of *trans*-2-benzoyloxymethyl-4-(N_4'-acetyl-cytosin-1'-yl)-1,3-oxathiolane (example 6) (63 mg) in methanolic ammonia (50 ml) was stirred overnight at

room temperature (16 hours). The solvent was removed *in vacuo* and the residue was solidified with ether to give 36 mg (93%) of the desired product which was characterized by ^{1}H-and ^{13}C-NMR.

m.p.: 175-177°C

UV: (CH_3OH) Lamda max: 270 nm

^{1}H-NMR: δ (ppm in DMSO d_6)

7.67 (d, 1H, C_6'-H)

7.19 (d, 2H, C_4'-NH_2)

6.30 (d, 1H, C_4-H)

5.77 (d, 1H, C_5'-H)

5.56 (t, 1H, C_2-CH_2OH)

5.23 (t, 1H, C_2-H)

4.18 (m, 2H, C_5-H)

3.61 (m, 1H, C_2-CH_2OH)

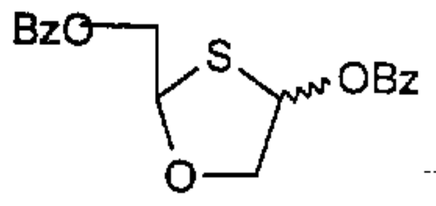
3.36 (m, 1H, C_2-CH_2OH)

^{13}C-NMR: δ (ppm in DMSO d_6)

166.00, 155.65, 142.30, 95.11, 87.52, 74.52, 63.42 and 62.86

Example 8

CIS AND _TRANS_-2-BENZOYLOXYMETHYL-4-BENZOYLOXY-1,3-OXATHIOLANE



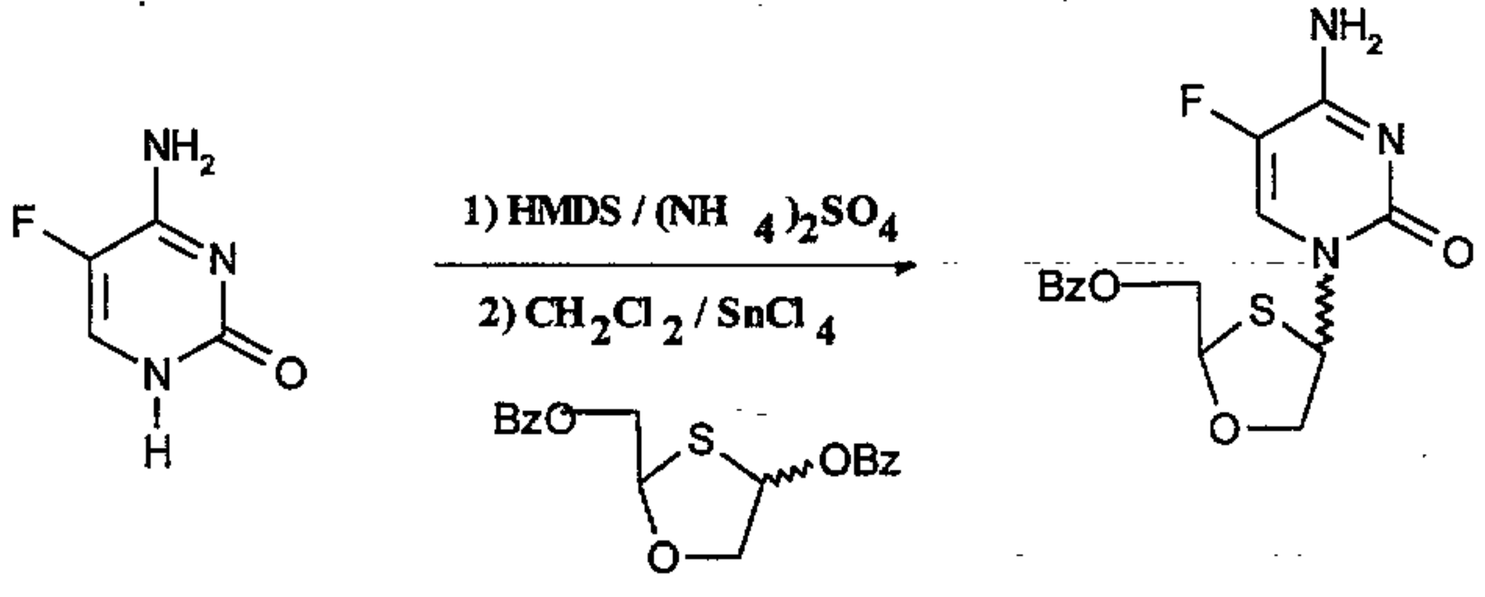
2-Benzoyloxymethyl-1,3-oxathiolane example 2) (0.4 g, 1.78 mmol) was dissolved in 200 mL of degased dry benzene under a stream of argon. To this solution was added benzoyl peroxide (0.863 g, 3.56 mmol) and AIBN catalyst (15 mg, 0.09 mmol, 5%) in one portion. The resulting mixture was refluxed for 4 hours under argon. The solvent was then removed under high vacuum, and the residue was dissolved in dichloromethane (40 ml), extracted 2X with a solution of 10% sodium bicarbonate (15 mL) and dried over $MgSO_4$. Evaporation of dichloromethane under reduced

pressure and purification of the residue on silica gel column using ethyl acetate; hexane (30%) as eluant afforded the pure mixture of *cis*- and *trans*-1,3-oxathiolane derivative as a colorless oil (264 mg, 0.76 mmol, 43% yield). The *trans* isomer was separated as a white solid m.p. 60-62°C. This mixture was fully characterized by ^{1}H, and ^{13}C NMR spectrum. R_f= 0.43 (ethyl acetate:hexanes 1:4).

1H NMR δ (ppm in $CDCl_3$): 8.07 (m, 2H, aromatic), 7.59 (m, 1H, aromatic), 7.56 (m, 2H, aromatic), 6.51 (t, 1H, C_4-H), 5.79 (m, 1H, C_2-H), 4.63 (m, 2H, CH_2-OCOPh), 4.32 (m, 2H, C_5-H). 13C NMR δ (ppm in $CDCl_3$): 166.56, 166.36, 134.12, 133.78, 130.35, 129.04, 84.40, 82.53, 75.04, 65.33, 39.89, 25.43, 23.93.

Example 9

CIS AND TRANS-2-BENZOYLOXYMETHYL-4-(5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE



5-fluorocytosine (700 mg, 5.4 mmol) was heated at reflux HMDS(1,1,1,3,3,3-hexamethyldisilazane, 30 ml) containing catalytic amount of ammonium sulfate (20 mg) for overnight (16 h). The clear solution was evaporated to dryness under reduced pressure and the residue was dissolved in dry methylene chloride (50 ml). To this solution was added through a cannula a mixture of 2-benzoyloxymethyl-4-benzoyloxy-1,3-oxathiolane (example 8) (1.25 g, 3.63 mmol) in dry methylene chloride (50 ml), followed by adding tin tetrachloride (4 ml of 1M solution

in methylene chloride). The reaction mixture was stirred under argon atmosphere at room temperature for 16h and heated at reflux for 1h. After cooling to room temperature the mixture was poured into saturated aqueous $NaHCO_3$ solution (150 ml), stirred for 15 min. and filtered over celite. The organic layer was collected. The aqueous solution was further extracted with methylene chloride (2 x 100 ml). The combined organic phase was washed twice with water (2 x 150 ml), once with brine solution, dried over Na_2SO_4, and filtered. Solvent was removed under reduced pressure. The residue contained two compounds in a ratio of 2:3 for *cis* and *trans* isomers and was purified on silica gel using ethyl acetate-methanol 95:5 as eluant to give 280 mg of *cis* isomer and 420 mg of *trans* isomer for a total yield of 53 %.

***Cis* isomer:**

Mp.: 235-236°C (dec.).

R_f. : 0.36 (EtOAc:MeOH 9:1)

^{1}H-NMR (300 MHz, DMSO-d_6): δ in ppm: 7.94 (m, 2H, aromatic), 7.80 (d, 2H, H-6' and 1H of NH_2 underneath, J_{H-F}=6.8 Hz), 7.68 (m, 1H, aromatic), 7.58 (b, 1H of NH_2), 7.47 (m, 2H, aromatic), 6.28 (d, 1H, H-4, J=3.0 Hz), 5.51 (t, 1H, H-2, J=3.8 Hz), 4.73 (m, 2H, -CH_2OBz), 4.61 (d, 1H, H-5, J=11 Hz) and 3.98 (dd, 1H, H-5, J=4.7 and 11 Hz).

***Trans*-isomer:**

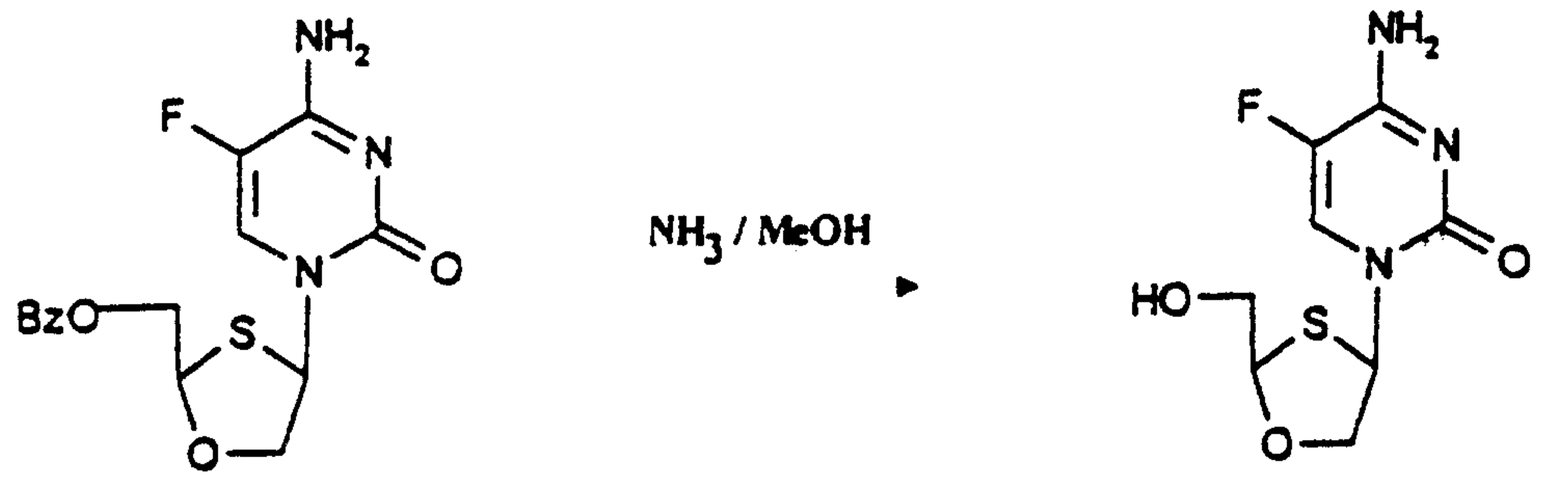
Mp.: 235-236°C (dec.).

^{1}H-NMR (300 MHz, DMSO-d_6): δ in ppm: 7.97 (m, 2H, aromatic), 7.81 (d, 2H, H-6' and 1H of NH_2 underneath, J_{H-F}=7.1 Hz), 7.66 (m, 1H, aromatic), 7.55 (m, 3H, 2H of aromatic and 1H of NH_2), 6.32 (d, 1H, H-4, J=4.8 Hz), 6.02 (dd, 1H, H-2, J=3.2 and 8.5 Hz), 4.55 (dd, 1H, -CH_2OBz, J=8.4 and 11.9 Hz), 4.38 (d, 1H, H-5, J=10.2 Hz) and 4.26 (dd, 2H, 1H of H-5 and 1H of -CH_2OBz, J=3.6 and 10.5 Hz).

74872-53

Example 10

CIS-2-HYDROXYMETHYL-4-(5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE (BCH-1081)



Cis-2-benzoyloxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane (example 9) (75 mg , 0.21 mmol) was dissolved in methanolic ammonia (25 ml). The mixture was stirred at room temperature for 16h and solvents were removed under reduced pressure. The residue was triturated with ether (2 x 20 ml). The remaining solid was recrystallized in ethanol-ether to give the desired compound (43 mg) in 80% yield.

M.p.: >210°C (dec.)

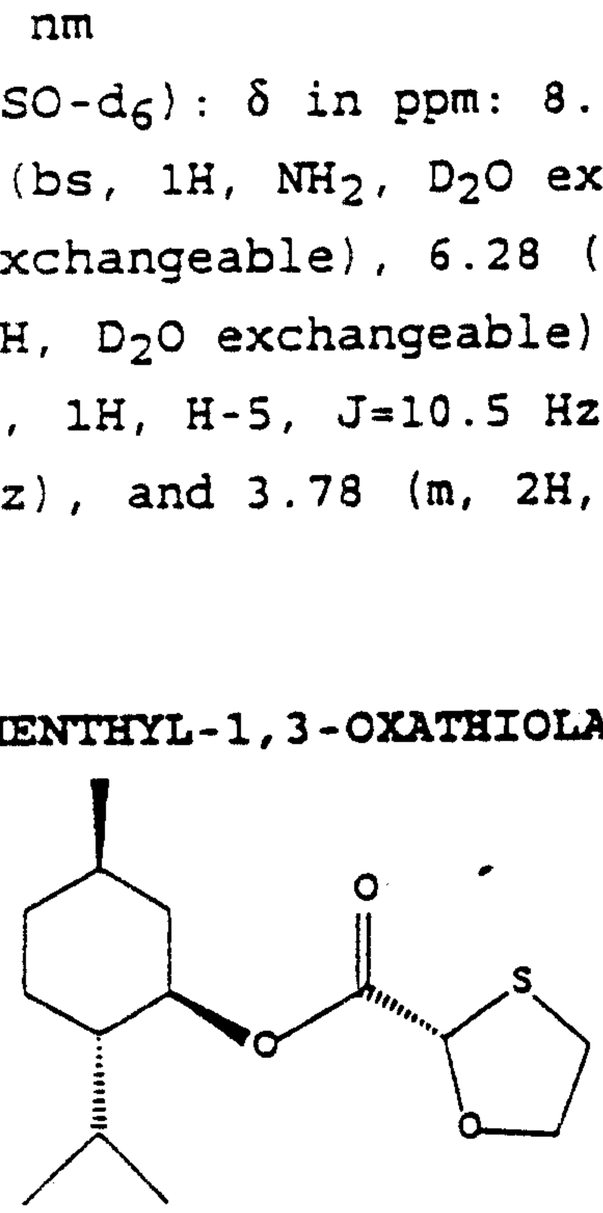
R_f: 0.41 (EtOAc:MeOH 4:1)

UV: λ_{max} (H_2O): 284 nm

^1H-NMR (300 MHz, DMSO-d_6): δ in ppm: 8.14 (d, 1H, H-6', J_{H-F}=7.1 Hz), 7.79 (bs, 1H, NH_2, D_2O exchangeable), 7.56 (bs, 1H, NH_2, D_2O exchangeable), 6.28 (d, 1H, H-4, J=2.6 Hz), 5.44 (t, 1H, OH, D_2O exchangeable), 5.18 (t, 1H, H-2, J=5.5 Hz), 4.44 (d, 1H, H-5, J=10.5 Hz), 3.91 (dd, 1H, H-5, J=4.6 and 10.6 Hz), and 3.78 (m, 2H, CH_2OH) .

Example 11

(1'R,2'S, 5'R)-MENTHYL-1,3-OXATHIOLAN-2S-CARBOXYLATE



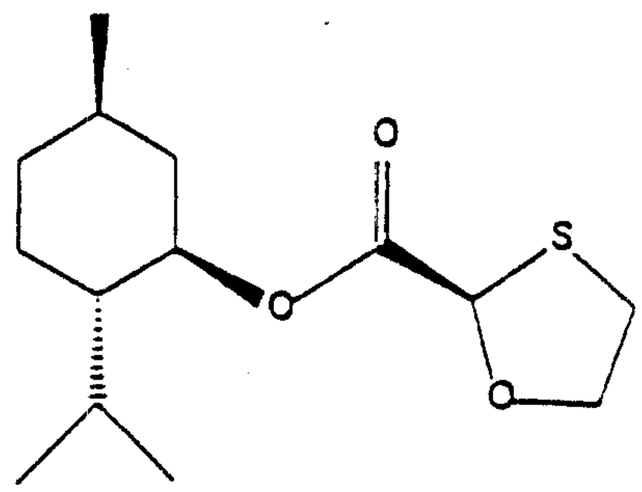
To a mixture of (1'R,2'S, 5'R)-menthyl-5S-acetoxy-1,3-oxathiolan-2S-carboxylate (WO 92/20669,

74872-53

(1.0 g, 3.03 mmol) and triethylsilane (4.84 ml, 30.3 mmol) at room temperature under an argon atmosphere was added trimethylsilyl trifluoromethanesulfonate (0.584 ml, 3.03 mmol). The reaction mixture was stirred at room temperature for 12h and then diluted with dichloromethane (150 ml), washed with saturated aqueous solution of $NaHCO_3$, water, brine, dried over sodium sulfate and concentrated. Chromatography (Hexane-EtOAc, 6:1) of the crude product gave the product (0.71g, 87%) as colorless oil: ^1H NMR in $CDCl_3$: δ 0.45-2.10 (m, 17H), 2.96-3.20 (m, 2H), 4.20-4.40 (m, 2H), 4.72 (dt, 1H), 5.45 (s, 1H); $[\alpha]_D^{25}$ - 102.9° (c, 1.02, $CHCl_3$).

Example 12

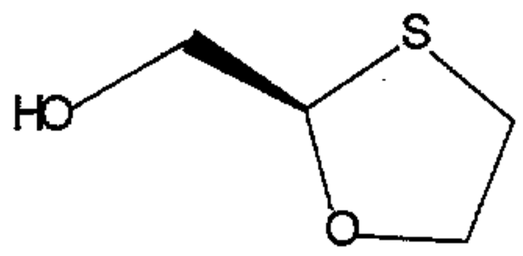
(1'R,2'S, 5'R)-MENTHYL-1,3-OXATHIOLAN-2R-CARBOXYLATE



To a mixture of (1'R,2'S, 5'R)-menthyl-5S-acetoxy-1,3-oxathiolan-2R-carboxylate (WO 92/20669) (0.50 g, 1.51 mmol) and triethylsilane (2.42 ml, 15.1 mmol) at room temperature under an argon atmosphere was added trimethylsilyl trifluoromethanesulfonate (0.29 ml, 1.51 mmol). The reaction mixture was stirred at room temperature for 12h and then diluted with dichloromethane (125 ml), washed with saturated aqueous solution of $NaHCO_3$, water, brine, dried over sodium sulfate and concentrated. Chromatography (Hexane-EtOAc, 6:1) of the crude product gave the product (0.369g, 86%) as colorless oil: ^1H NMR in $CDCl_3$: δ 0.40-2.10 (m, 17H), 2.98-3.19 (m, 2H), 4.20-4.40 (m, 2H), 4.72 (dt, 1H), 5.46 (s, 1H).

Example 13

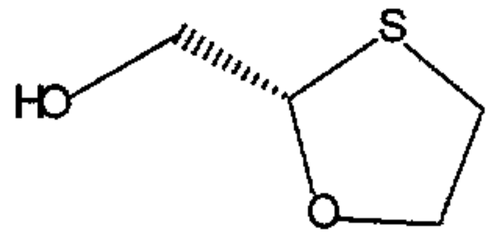
2R-HYDROXYMETHYL-1,3-OXATHIOLANE



To a solution of (1'R,2'S, 5'R)-menthyl-1,3-oxathiolan-2R-carboxylate (example 12) (3.23 g, 11.9 mmol) in anhydrous ethanol (20 ml) at 0°C under an argon atmosphere were added sodium borohydride (1.12 g, 29.7mmol) and anhydrous methanol (0.916 ml, 47.6 mmol). The reaction mixture was stirred at 0°C for 1h and allowed to warm to room temperature and stirred for 12h. The reaction was then quenched with acetic acid and the solvent was removed *in vacuo*. The obtained residue was diluted with dichloromethane (225 ml), washed with water, brine, dried over sodium sulfate and concentrated. Chromatography (Hexane-Et_2O, 1:1) of the crude product gave the product (1.30g, 91%) as colorless oil: ^1H NMR in $CDCl_3$ δ 2.85-2.97 (m, 2H), 3.58 (dd, 1H, J=12.2, 5.4Hz), 3.66 (dd, 1H, J=12.2, 3.3Hz), 3.75-3.85 (m, 1H), 4.14-4.25 (m, 1H), 5.16 (dd, 1H, J=5.4, 3.3Hz)

Example 14

2S-HYDROXYMETHYL-1,3-OXATHIOLANE

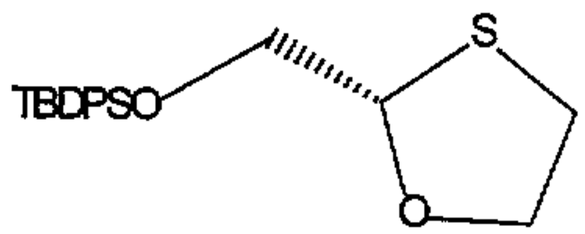


To a solution of (1'R,2'S, 5'R)-menthyl-1,3-oxathiolan-2S-carboxylate (example 11) (1.16 g, 4.26 mmol) in anhydrous ethanol (10 ml) at 0°C under an argon atmosphere were added sodium borohydride (0.403 g, 10.7mmol) and anhydrous methanol (0.690 ml, 17.03 mmol). The reaction mixture was stirred at 0°C for 1h and allowed to warm to room temperature and stirred for 12h. The reaction was then quenched with acetic acid and the solvent was removed *in vacuo*. The obtained residue was

diluted with dichloromethane (150 ml), washed with water, brine, dried over sodium sulfate and concentrated. Chromatography (Hexane-Et_2O, 1:1) of the crude product gave the product (0.47 g, 92%) as colorless oil: ^1H NMR in $CDCl_3$: δ 2.85-2.99 (m, 2H), 3.60 (dd, 1H, J=12.2, 5.5Hz), 3.65 (dd, 1H, J=12.2, 3.3Hz), 3.80-3.90 (m, 1H), 4.20-4.30 (m, 1H), 5.15 (dd, 1H, J=5.5, 3.3Hz); $[\alpha]_D^{25}$ -35.6° (c, 1.25, $CHCl_3$).

Example 15

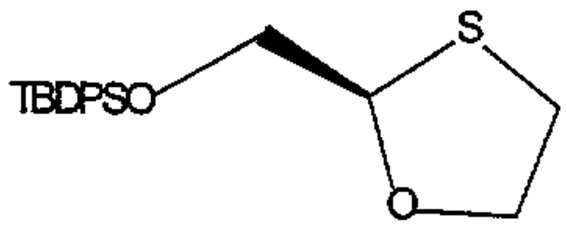
2S-t-BUTYLDIPHENYLSILYLOXYMETHYL-1,3-OXATHIOLANE



To a solution of 2S-hydroxymethyl-1,3-oxathiolane (example 14) (0.63 g, 5.3 mmol), imidazole (0.71 g, 10.4 mmol) in tetrahydrofuran (15 ml) at 0°C under an argon atmosphere was added a solution of t-butyldiphenylsilyl chloride (2.16g, 7.9 mmol) in tetrahydrofuran (8 ml). The reaction mixture was allowed to warm to room temperature and stirred for 12h then diluted with dichloromethane (125 ml). The organic layer was washed with water, brine, dried over sodium sulfate and concentrated. Chromatography (Hexane-EtOAc, 6:1) of the crude product gave the product (1.87g, 99 %) as colorless oil: ^1H NMR in $CDCl_3$: δ 1.08 (s, 9H), 2.93-2.99 (m, 2H), 3.70 (dd, 1H, J=10.9, 4.6Hz), 3.86 (dd, 1H, J=10.9, 6.4Hz), 3.94-4.15 (m, 2H), 5.29 (dd, 1H, J=6.4, 4.6Hz), 7.35-7.50 (m, 6H), 7.68-7.75 (m, 4H), $[\alpha]_D^{25}$ -23.3° (c, 1.0, $CHCl_3$).

Example 16

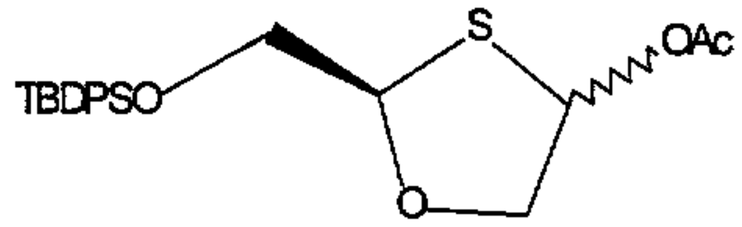
2R-t-BUTYLDIPHENYLSILYLOXYMETHYL-1,3-OXATHIOLANE



To a solution of 2R-hydroxymethyl-1,3-oxathiolane (example 13) (1.30 g, 10.8 mmol), imidazole (1.47 g, 21.6 mmol) in tetrahydrofuran (30 ml) at 0°C under an argon atmosphere was added a solution of t-butyldiphenylsilyl chloride (4.46g, 16.2 mmol) in tetrahydrofuran (15 ml). The reaction mixture was allowed to warm to room temperature and stirred for 12h, then diluted with dichloromethane (150 ml). The organic layer was washed with water, brine, dried over sodium sulfate and concentrated. Chromatography (Hexane-EtOAc, 6:1) of the crude product gave the product (3.64 g, 94 %) as colorless oil: ^1H NMR in $CDCl_3$: δ 1.04 (s, 9H), 2.89-2.99 (m, 2H), 3.65 (dd, 1H, J=10.9, 4.6Hz), 3.88 (dd, 1H, J=10.9, 6.4Hz), 3.90-4.14 (m, 2H), 5.27 (dd, 1H, J=6.4, 4.6Hz), 7.34-7.46 (m, 6H), 7.64-7.74 (m, 4H).

Example 17

TRANS AND _CIS_-2R-t-BUTYLDIPHENYLSILYLOXYMETHYL-4-ACETOXY-1,3-OXATHIOLANE

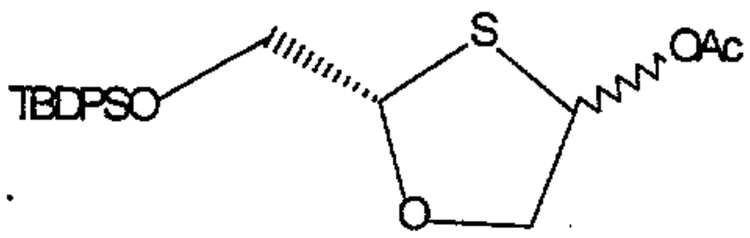


Solid meta-chloroperbenzoic acid (MCPBA) (0.23 g, 80%, 1.06 mmol) was added to a stirred solution of 2R-t-butyldiphenylsiloxymethyl-1,3-oxathiolane (example 16) (0.32g, 0.89 mmol) in dichloromethane (20 ml) at 0°C. The reaction mixture was stirred at 0°C for 2h and then diluted with dichloromethane (150 ml), washed with saturated aqueous Na_2CO_3 solution, water, brine, dried over sodium sulfate and concentrated. Chromatography of the crude product gave a mixture of sulfoxides. The mixture of the obtained sulfoxide, acetic anhydride (10 ml), tetrabutyl ammonium acetate (0.32 g , 1.06 mmol) was then heated at 120°C for 6h and the excess acetic anhydride was removed *in vacuo*. The residue was diluted in dichloromethane (150 ml), washed with water, brine, dried over sodium sulfate and concentrated. Chromatography

of the crude product gave a mixture of acetate (0.164 g, 45%) as colorless oil: ^{1}H NMR in $CDCl_3$: δ 1.05 (s, 9H), 2.03 (s, 1.35H), 2.08 (s, 1.65H), 3.60-4.50 (m, 4H), 5.28 (t, 0.45H, J=5.4 Hz), 5.55 (dd, 0.55H, J=6.5, 4.7Hz), 6.14-6.20 (m, 1H), 7.30-7.50 (m, 6H), 7.60-7.78 (m, 4H).

Example 18

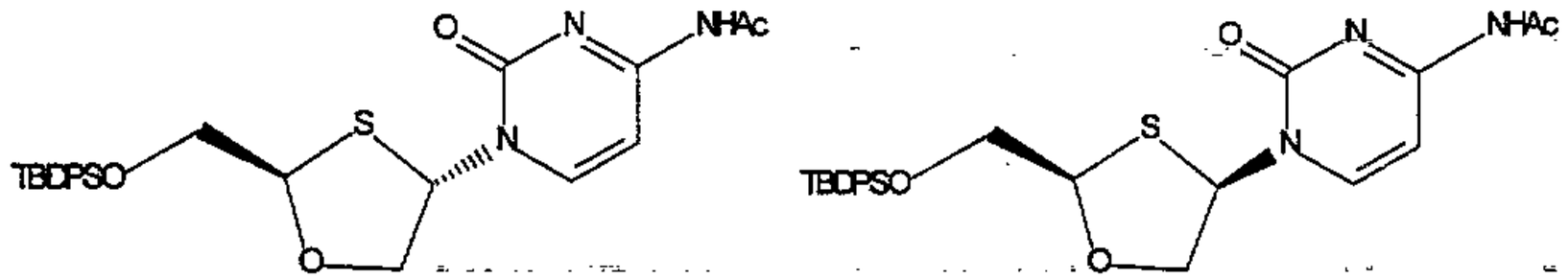
***TRANS* AND *CIS*-2S-t-BUTYLDIPHENYLSILYLOXYMETHYL-4-ACETOXY-1,3-OXATHIOLANE**



Solid MCPBA (0.85 g, 80%, 3.9 mmol) was added to a stirred solution of 2S-t-butyldiphenylsiloxymethyl-1,3-oxathiolane (example 15) (1.35g, 3.9 mmol) in dichloromethane (30 ml) at 0°C. The reaction mixture was stirred at 0°C for 2h and then diluted with dichloromethane (225 ml), washed with saturated aqueous Na_2CO_3 solution, water, brine, dried over sodium sulfate and concentrated. Chromatography of the crude product gave a mixture of sulfoxides. The mixture of the obtained sulfoxide, acetic anhydride (15 ml), tetrabutyl ammonium acetate (1.19g , 3.9 mmol) was then heated at 120°C for 6h and the excess acetic anhydride was removed *in vacuo*. The residue was diluted with dichloromethane (200 ml), washed with water, brine, dried over sodium sulfate and concentrated. Chromatography of the crude product gave a mixture of acetate (0.601 g, 40%) as colorless oil: ^{1}H NMR in $CDCl_3$: δ 1.10 (s, 9H), 2.05 (s, 1.2H), 2.10 (s, 1.8H), 3.60-4.50 (m, 4H), 5.30 (t, 0.4H, J=5.5 Hz), 5.58 (dd, 0.6H, J=6.5, 4.8Hz), 6.14-6.23 (m, 1H), 7.33-7.50 (m, 6H), 7.65-7.78 (m, 4H).

Example 19

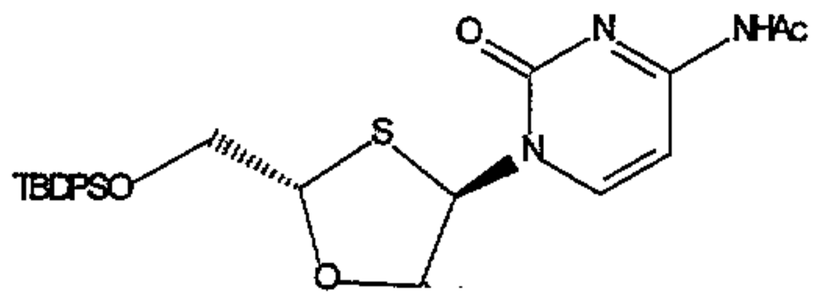
2R-t-BUTYLDIPHENYLSILYLOXYMETHYL-4S-(N-4'-ACETYLCYTOSIN-1'-YL)-1,3-OXATHIOLANE AND 2R-t-BUTYLDIPHENYLSILYLOXYMETHYL-4R-(N-4'-ACETYLCYTOSIN-1'-YL)-1,3-OXATHIOLANE

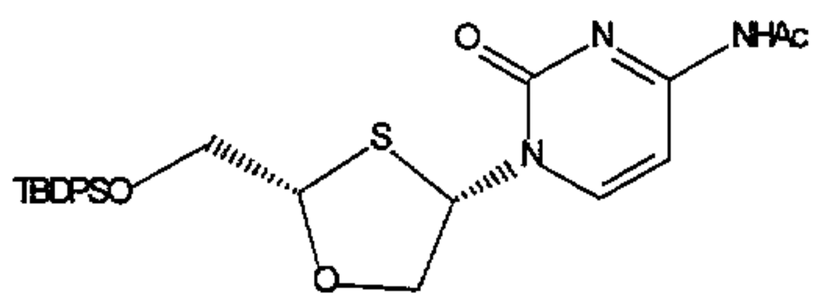


2,6-lutidine (0.054 ml, 0.49 mmol) and trimethylsilyl trifluoromethanesulfonate (0.095 ml, 0.49 mmol) were added to a suspension of N-4'-acetylcytosine (0.045 g, 0.29 mmol) in dichloroethane (2 ml) at room temperature under argon atmosphere. The mixture was stirred for 15 min and mixture of *cis* and *trans* 2R-t-butyldiphenylsiloxymethyl-4-acetoxy-1,3-oxathiolane (example 17) (0.100 g, 0.25 mmol) in dichloroethane (2 ml) and trimethylsilyl trifluoromethanesulfonate (0.048 ml, 0.25 mmol) were introduced successively. The reaction mixture was heated to reflux for half hour and diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ solution, water, brine, dried over sodium sulfate and concentrated. Chromatography of the crude product gave a mixture of *trans* and *cis* isomer which was subjected to preparative TLC separation to give *cis* isomer (33 mg, 26%) [^{1}H NMR in $CDCl_3$: δ 1.08 (s, 9H), 2.22 (s, 3H), 3.85-4.45 (m, 4H), 5.26 (t, 1H, J=4.3Hz), 6.54 (d, 1H, J=4.2Hz), 7.21 (d,1H, J=7.4Hz), 7.35-7.50 (m, 6H), 7.60-7.75 (m, 4H), 8.23 (d, 1H, J=7.4Hz), 9.02 (bs,1H)] and *trans* isomer (49 mg, 39%) [^{1}H NMR in $CDCl_3$: δ 1.04 (s, 9H), 2.21 (s, 3H), 3.56-4.26 (m, 4H), 5.64 (dd, 1H, J=6.8, 4.4Hz), 6.39 (d, 1H, J=3.6Hz), 7.31-7.48 (m, 7H), 7.58-7.71 (m, 4H), 8.01 (d, 1H, J=7.4Hz), 8.86 (bs, 1H)].

Example 20

2S-t-BUTYLDIPHENYLSILYLOXYMETHYL-4R-(N-4'-ACETYLCYTOSIN-1'-YL)-1,3-OXATHIOLANE AND 2S-t-BUTYLDIPHENYLSILYLOXY METHYL-4S-(N-4'-ACETYLCYTOSIN-1'-YL)-1,3-OXATHIOLANE

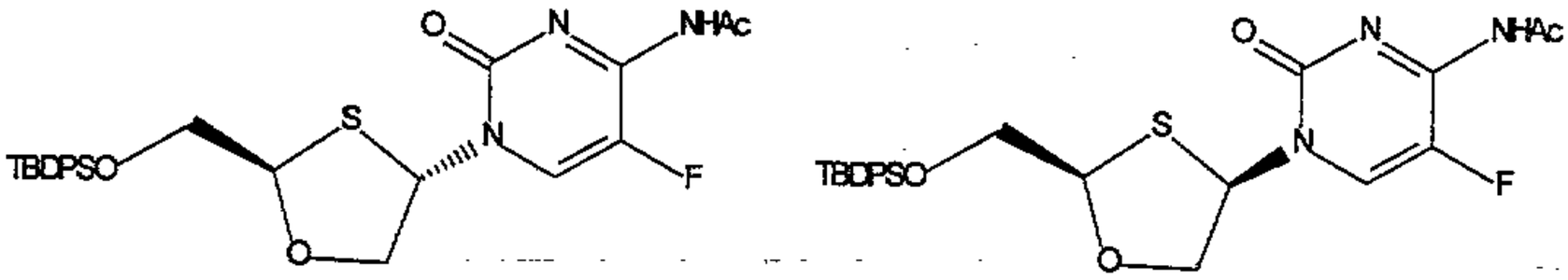




2,6-lutidine (0.131 ml, 1.13 mmol) and trimethylsilyl trifluoromethanesulfonate (0.218 ml, 1.13 mmol) were added to a suspension of N-4'-acetylcytosine (0.104 g, 0.68 mmol) in dichloroethane (2 ml) at room temperature under argon atmosphere. The mixture was stirred for 15 min and mixture of *cis* and *trans* 2S-t-butyldiphenylsiloxymethyl-4-acetoxy-1,3-oxathiolane (example 18) (0.235 g 0.56 mmol) in dichloroethane (2 ml) and trimethylsilyl trifluoromethanesulfonate (0.109 ml, 1.13 mmol) were introduced successively. The reaction mixture was heated to reflux for half hour and diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ solution, water, brine, dried over sodium sulfate and concentrated. Chromatography (EtOAc) of the crude product gave a mixture of *trans* and *cis* isomer (0.225 g, 78 %): ^1H NMR in $CDCl_3$: δ 1.05 (s, 5.4H), 1.08 (s, 3.6H), 2.24 (s, 1.2H), 2.28 (s, 1.8H), 3.67-4.42 (m, 4H), 5.26 (t, 0.4H, J=4.3Hz), 5.64 (dd, 0.6H, J=6.8, 4.3Hz), 6.40 (d, 0.6H, J=3.8Hz), 6.53 (d, 0.4H, J=4.1Hz), 7.20-7.75 (m, 11H), 8.00 (d, 0.6H, J=7.5Hz), 8.21 (d, 0.4H, J=7.5Hz), 9.63 (bs,1H).

Example 21

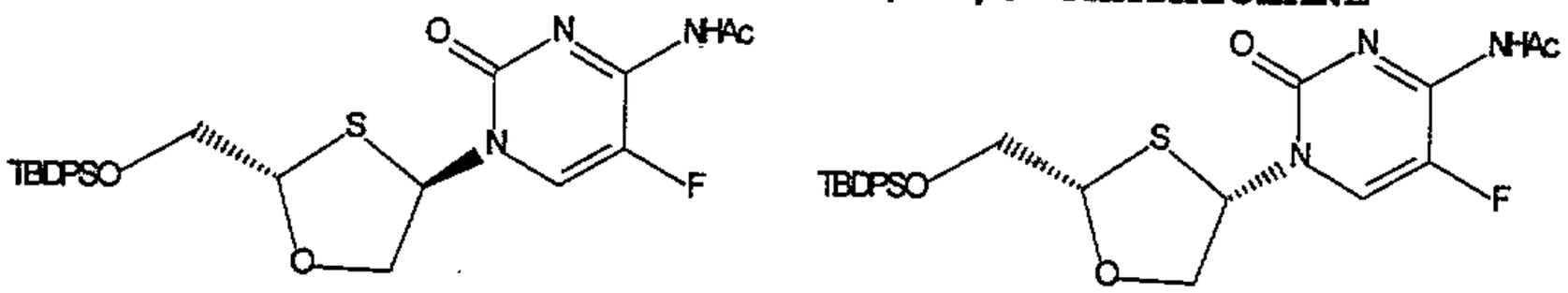
2R-t-BUTYLDIPHENYLSILYLOXYMETHYL-4S-(N-4'-ACETYL-5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE AND 2R-t-BUTYLDIPHENYLSILYLOXYMETHYL-4R-(N-4'-ACETYL-5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE



2,6-lutidine (0.124 ml, 1.07 mmol) and trimethylsilyl trifluoromethanesulfonate (0.207 ml, 1.07 mmol) were added to a suspension of. N-4'-acetyl-5'-fluorocytosine (0.110 g, 0.64 mmol) in dichloroethane (3 ml) at room temperature under argon atmosphere. The mixture was stirred for 15 min and mixture of *cis* and *trans* 2R-t-butyldiphenylsiloxymethyl-4-acetoxy-1,3-oxathiolane (example 17) (0.223 g 0.54 mmol) in dichloroethane (3 ml) and trimethylsilyl trifluoromethane-sulfonate (0.103 ml, 0.54 mmol) were introduced successively. The reaction mixture was heated to reflux for half hour and diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ solution, water, brine, dried over sodium sulfate and concentrated. Chromatography of the crude product gave a mixture of *trans* and *cis* isomer which was subjected to preparative TLC separation to give *cis* isomer (97 mg, 34%) [^1H NMR in $CDCl_3$ δ 1.08 (s, 9H), 2.65 (s, 3H), 3.90-4.50 (m, 4H), 5.27 (t, J=4.2Hz), 6.53 (d, 1H, J=4.2Hz), 7.32-7.530 (m, 6H), 7.55-7.76 (m, 4H), 8.21 (d, 1H, J=6.1Hz)] and *trans* isomer (68 mg, 24%) [^1H NMR in $CDCl_3$ δ 1.07 (s, 9H), 2.63 (s, 3H), 3.56-4.26 (m, 4H), 5.68 (dd, J=6.9, 4.4Hz), 6.37 (d, 1H, J=2.4Hz), 7.30-7.53 (m, 6H), 7.57-7.75 (m, 4H), 7.93 (d, 1H, J=6.1Hz)].

Example 22

2S-t-BUTYLDIPHENYLSILYLOXYMETHYL-4R-(N-4'-ACETYL-5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE AND 2S-t-BUTYLDIPHENYLSILYLOXYMETHYL-4S-(N-4'-ACETYL-5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE

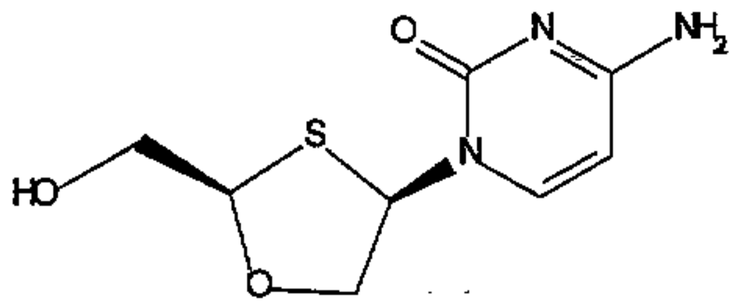


2,6-lutidine (0.248 ml, 2.13 mmol) and trimethylsilyl trifluoromethanesulfonate (0.418 ml, 2.13 mmol) were added to a suspension of N-4'-acetyl-5'-fluorocytosine (0.218 g, 1.27 mmol) in dichloroethane (4.5 ml) at room temperature under argon atmosphere. The mixture was stirred for 15 min and mixture of *cis* and *trans* 2S-t-butyldiphenylsiloxymethyl-4-acetoxy-1,3-oxathiolane (example 18) (0.433g 1.06 mmol) in dichloroethane (4 ml) and trimethylsilyl trifluoromethane-sulfonate (0.206 ml, 1.06 mmol) were introduced successively. The reaction mixture was heated to reflux for half hour and diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ solution, water, brine, dried over sodium sulfate and concentrated. Chromatography of the crude product gave a mixture of *trans* and *cis* isomer which was subjected to preparative TLC separation to give *cis* isomer (145 mg, 26%) [^1H NMR in $CDCl_3$ δ 1.09 (s, 9H), 2.66 (s, 3H), 3.90-4.50 (m, 4H), 5.26 (t, J=4.1Hz), 6.53 (d, 1H, J=4.1Hz), 7.36-7.50 (m, 6H), 7.55-7.78 (m, 4H), 8.20 (d, 1H, J=6.1Hz)] and *trans* isomer (119 mg, 21%) [^1H NMR in $CDCl_3$ δ 1.07 (s, 9H), 2.64 (s, 3H), 3.58-4.25 (m, 4H), 5.68 (dd, J=6.8, 4.2Hz), 6.37 (d, 1H, J=2.7Hz), 7.35-7.51 (m, 6H), 7.60-7.76 (m, 4H), 7.93 (d, 1H, J=6.1Hz)].

Example 23

2R-HYDROXYMETHYL-4R-(CYTOSIN-1'-YL)-1,3-OXATHIOLANE

COMPOUND #1



To a solution of 2R-t-butyldiphenylsiloxymethyl-4R-(N-4'-acetylcytosin-1'-yl)-1,3-oxathiolane (example 19) (72 mg, 0.14 mmol) in THF (3 ml) at ambient temperature under an argon atmosphere were slowly added tetrabutylammonium fluoride solution (0.212 ml, 1M in THF, 0.21 mmol) and glacial acetic acid (0.012 ml, 0.21 mmol). The reaction mixture was allowed to stir for 1h, followed by the addition of silica gel (0.5 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 4:1) to give the desilylated product. The desilylated product was dissolved in saturated K_2CO_3 methanol solution and the reaction mixture was stirred at ambient temperature for half an hour. The reaction mixture was neutralized with 1N HCl/methanol solution followed by the addition of silica gel (0.5 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 4:1) to afford the product (27 mg, 91%) as white solid which was triturated in Et_2O-MeOH: mp 200°C (dec.); $[\alpha]_D^{25}$ -126.8° (c, 0.5, MeOH); ^1H NMR (DMSO-d_6): δ 3.70-3.82 (m, 2H), 3.91 (dd,1H, J=10.4, 4.6Hz), 4.38 (d, 1H, J=10.4Hz), 5.16 (t, 1H, J=4.6Hz), 5.32 (t, 1H, J=5.8Hz), 5.75 (d, 1H, J=7.4Hz), 6.32 (d,1H, J=4.6Hz), 7.12 (bs, 1H), 7.23 (bs, 1H), 7.84 (d,1H, J=7.4Hz); ^{13}C NMR (DMSO-d_6): δ 62.7, 63.1, 77.4, 89.0, 95.6, 142.4, 155.4, 165.8.

In order to assess the enantiomeric purity of the synthesized end nucleoside product or to resolve the racemic mixture of deprotected nucleoside analogues chiral

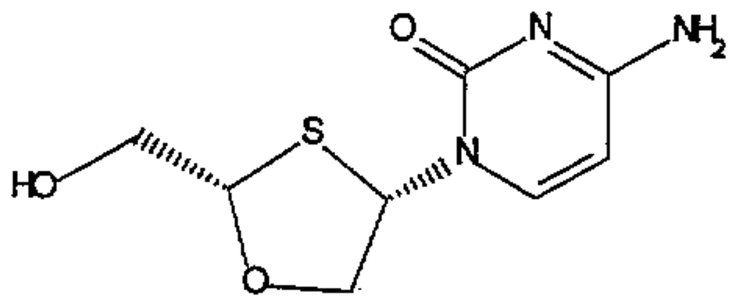
HPLC methods were used. For example, the compound #1 above, was found to have a retention time of 43.88 min under the following conditions:

Type of column:	cyclobond™ RSP 4.6x250nm
Flow rate:	0.27ml/min
Solvent:	0.05% TFAA, PH 7.0
Detection:	254nm

Example 24

2S-HYDROXYMETHYL-4S-(CYTOSIN-1'-YL)-1,3-OXATHIOLANE

COMPOUND #2



To a solution of 2S-t-butyldiphenylsiloxymethyl-4S-(N-4'-acetylcytosin-1'-yl)-1,3-oxathiolane (example 20) (202 mg, 0.40 mmol) in THF (3.5 ml) at ambient temperature under an argon atmosphere were slowly added tetrabutylammonium fluoride solution (0.595 ml, 1M in THF, 0.60 mmol) and glacial acetic acid (0.034 ml, 0.60 mmol). The reaction mixture was allowed to stir for 1h, followed by the addition of silica gel (0.9 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 9:1) to give the desilylated product. The desilylated product was dissolved in saturated K_2CO_3 methanol solution and the reaction mixture was stirred at ambient temperature for half an hour. The reaction mixture was neutralized with 1N HCl methanol solution followed by the addition of silica gel (0.9 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 4:1) to afford the product (74 mg, 81%) as white solid which was triturated in Et_2O-MeOH: mp 220°C (dec.); $[\alpha]_D^{25}$ +118.6° (c, 0.5, MeOH); ^1H NMR (DMSO-d_6): δ 3.70-3.82 (m, 2H), 3.92 (dd,1H, J=10.6, 4.6Hz), 4.38 (d, 1H, J=10.6Hz), 5.17 (t, 1H, J=4.5Hz),

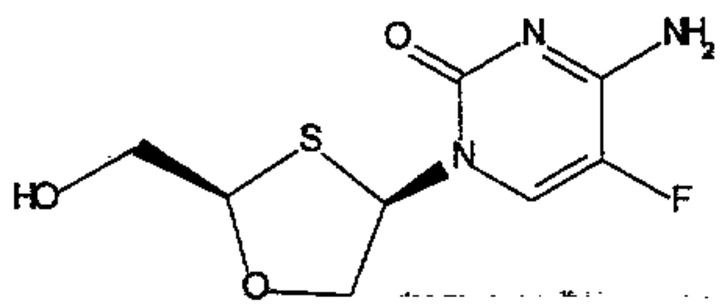
5.32 (t, 1H, J=5.8Hz), 5.75 (d, 1H, J=7.4Hz), 6.32 (d,1H, J=4.6Hz), 7.12 (bs, 1H), 7.22 (bs, 1H), 7.85 (d,1H, J=7.4Hz); ^{13}C NMR (DMSO-d_6): δ 63.0, 63.1, 77.4, 88.9, 95.1, 142.4, 155.4, 165.7.

In order to assess the enantiomeric purity of the synthesized end nucleoside product or to resolve the racemic mixture of deprotected nucleoside analogues chiral HPLC methods were used. For example, the compound **#2** above, was found to have a retention time of 48.18 min under the following conditions:

Type of column:	cyclobond™ RSP 4.6x250nm
Flow rate:	0.27ml/min
Solvent:	0.05% TFAA, PH 7.0
Detection:	254nm

Example 25

2R-HYDROXYMETHYL-4R-(5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE - COMPOUND #3



To a solution of 2R-t-butyldiphenylsiloxymethyl-4R-(N-4'-acetyl-5'-fluorocytosin-1'-yl)-1,3-oxathiolane (example 21) (152 mg, 0.29 mmol) in THF (3.5 ml) at ambient temperature under an argon atmosphere were slowly added tetrabutylammonium fluoride solution (0.432 ml, 1M in THF, 0.43 mmol) and glacial acetic acid (0.025 ml, 0.43 mmol). The reaction mixture was allowed to stir for 1h, followed by the addition of silica gel (0.5 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 9:1) to give the desilylated product. The desilylated product was dissolved in saturated K_2CO_3 methanol solution and the reaction mixture

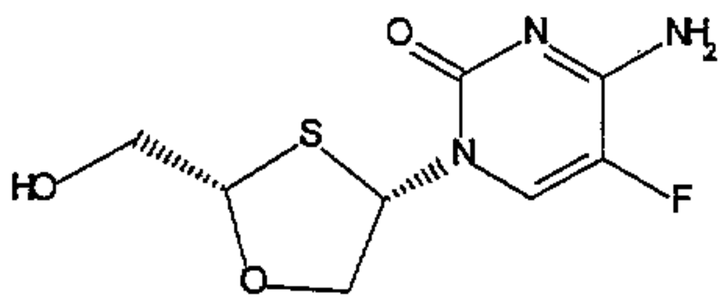
was stirred at ambient temperature for half an hour. The reaction mixture was neutralized with 1N HCl methanol solution followed by the addition of silica gel (0.5 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 4:1) to afford the product (58 mg, 81%) as white solid which was triturated in Et_2O-MeOH: mp 183°C (dec.); $[\alpha]_D^{25}$ -86.5° (c, 0.57, MeOH); ^1H NMR (DMSO-d_6): δ 3.70-4.50 (m, 4H), 5.18 (t, 1H, J=3.6Hz), 5.44 (t, 1H, J=5.8Hz), 6.25-6.30 (m,1H), 7.56 (bs, 1H), 7.80 (bs, 1H), 8.14 (d,1H, J=7.2Hz); ^{13}C NMR (DMSO-d_6): δ 62.2, 63.4, 77.5, 88.9, 126.7, 127.1, 134.7, 137.9, 153.8, 157.5, 157.7.

In order to assess the enantiomeric purity of the synthesized end nucleoside product or to resolve the racemic mixture of deprotected nucleoside analogues chiral HPLC methods were used. For example, the compound **#3** above, was found to have a retention time of 16.73 min under the following conditions:

Type of column:	cyclobond™ RSP 4.6x250nm
Flow rate:	0.43ml/min
Solvent:	0.05% TFAA, PH 7.0
Detection:	254nm

Example 26

2S-HYDROXYMETHYL-4S-(5'-FLUOROCYTOSIN-1'-YL)-1,3-OXATHIOLANE - COMPOUND #4



To a solution of 2S-t-butyldiphenylsiloxymethyl-4S-(N-4'-acetyl-5'-fluorocytosin-1'-yl)-1,3-oxathiolane (example 22) (78 mg, 0.15 mmol) in THF (3 ml) at ambient temperature under an argon atmosphere were slowly added

tetrabutylammonium fluoride solution (0.222 ml, 1M in THF, 0.22 mmol) and glacial acetic acid (0.013 ml, 0.22 mmol). The reaction mixture was allowed to stir for 1h, followed by the addition of silica gel (0.5 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 9:1) to give the desilylated product. The desilylated product was dissolved in saturated K_2CO_3 methanol solution and the reaction mixture was stirred at ambient temperature for half hour. The reaction mixture was neutralized with 1N HCl methanol solution followed by the addition of silica gel (0.5 g). The resulting slurry was subjected to silical gel column chromatography (EtOAc-MeOH, 4:1) to afford the product (36 mg, 98%) as white solid which was triturated in Et_2O-MeOH: mp 180°C (dec.); $[\alpha]_D^{25}$ +75.7° (c, 0.56, MeOH); ^1H NMR (DMSO-d_6): δ 3.69-4.50 (m, 4H), 5.18 (t, 1H, J=3.7Hz), 5.44 (t, 1H, J=5.8Hz), 6.25-6.29 (m,1H), 7.55 (bs, 1H), 7.8 (bs, 1H), 8.14 (d,1H, J=7.2Hz); ^{13}C NMR (DMSO-d_6): 61.8, 63.1, 77.1, 88.6, 126.3, 126.8, 134.4, 137.6, 153.5, 157.1, 157.3.

In order to assess the enantiomeric purity of the synthesized end nucleoside product or to resolve the racemic mixture of deprotected nucleoside analogues chiral HPLC methods were used. For example, the compound **#4** above, was found to have a retention time of 15.07 min under the following conditions:

Type of column:	cyclobond™ RSP 4.6x250nm
Flow rate:	0.43ml/min
Solvent:	0.05% TFAA, PH 7.0
Detection:	254nm

Example 27

ANTIVIRAL ACTIVITY:

FORMAZAN ASSAY ON MT-4 AND 3TC™-RESISTANT CELL LINES

Anti-HIV-1 antiviral activity was determined in MT-4 cells and MT-4 cells that have been made resistant to 3TC™. A suspension of cells (approximately 10^6 cells/ml) in RPMI 1640 growth medium was infected with HIV-1 strain RF at a M.O.I. of 10^{-3} infectious units/cell. An uninfected cell suspension was prepared in parallel to evaluate drug-induced cytotoxicity. The two suspensions were incubated for 90 minutes at room temperature. Test compounds were serially diluted in 10-fold decrements from 100 μg/ml to 0.01 μg/ml (final concentrations in two 96 well microtitre plates. 20 μl of infected cell suspension were inoculated into each well of one of the plates (anti-viral), while 20 μl of uninfected cell suspension were added to each well of the second plate (cytotoxicity). The plates were then incubated for 7 days at 37°C. After incubation, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 20 mg/ml was added to all wells and the plates incubated for a further 90 minutes at 37°C.

150 μl of 10% (v/v) alcoholic Triton X-100 was then added and the cells resuspended. After 15 minutes at room temperature, the plates were analyzed in a Multiskan MC reader at 405 nm. Conversion of yellow MMT to its formazan derivative is maximum in uninfected cells, and absent in untreated infected cells. The optical density values for the cytotoxicity controls and the antiviral test wells were graphically plotted and the dose of compounds required to inhibit the conversion of MMT to 50% of the untreated uninfected controls was calculated. In this way, both the 50% cytotoxic dose (CD 50%) and the 50% anti-viral dose (ID 50%) can be calculated. Table 1 shows CD 50% and ID 50% values obtained for:

TABLE 1 (MT-4 assay)

COMPOUND	ID_{50} (μg/ml)	CD_{50} (μg/ml)
BCH - 270 (example 7)	4.0	>100
#1 (example 23)	13	>100
#2 (example 24)	4.4	>100
#3 (example 25)	6.0	>100
#4 (example 26)	2.9	100
AZT (reference)	0.01	>1

TABLE 2 3TC-RESISTANT CELLS (Met^{184}→Ile mutation)

COMPOUND	ID_{50} (μg/ml)	CD_{50} (μg/ml)
#1 (example 23)	8.5	>100
#2 (example 24)	42	>100
#3 (example 25)	3.4	>100
#4 (example 26)	3.0	100
3TC™	>100	>100

INHIBITION OF SYNCYTIUM FORMATION

C8166 cells were infected with HIV-1 (strain RF) at a moil of $1x10^{-3}$ infectious units/cell and adsorbed at room temperature for 60 minutes. After adsorption, the cells were washed three times in growth medium. Aliquots of 10^5 cells were added to each well of 24-well plates containing serial dilution of test compounds at final concentrations of 50μg/ml to 0.05μg/ml in RPMI® 1640 growth medium. Untreated infected cells and untreated uninfected cells were also included as controls. The plates were incubated at 37°C/5% CO_2 for 3-4 days in humidified containers. The

cells were examined daily for evidence of HIV-1 induced syncytium formation. The syncytia were quantified by reference to the untreated infected controls, and the dose of compound required to reduce the cytopathic effect by 50% (ID_{50}) was calculated.

TABLE 3 (SYNCYTIUM FORMATION)

COMPOUND	ID_{50} (µg/ml)	CD_{50} (µg/ml)
#2 (example 25)	0.08	>100
#3 (example 25)	0.17	>100
#4 (example 26)	0.03	>100

CBM ASSAY.

Assays on cord blood mononuclear cells were done substantially as described in Gu et al., J. Virol. (1992), **66**:7128-7135.

In short, viruses (HTLV-III_B) that had initially been grown on MT-4 cells were passaged onto phytohemaglutinin-prestimulated cord blood mononuclear cells (CBM). For subsequent analysis, samples of CBM (5 x 10^5 cells per mL) were pretreated with various concentrations of the different compounds for 4 h and were then inoculated with CBL-grown HIV-1 at a multiplicity of infection of 1.0 in the concentration of the compound used for the pretreatment. Fresh medium, including the appropriate concentration of the compound was added three times weekly, and fresh phytohemaglutinin-prestimulated CBM (5 x 10^5 cells per mL) were added at 2-day intervals. The calculation of IC_{50} was determined on the basis of RT levels in culture fluids as described in Gao et al., (1992), J. Virol. **66**: 12-19.

TABLE 4 (CBM)

COMPOUND	ID_{50} (µg/ml)	CD_{50} (µg/ml)
BCH - 270 (example 7)	0.03	>23
#1 (example 23)	0.05-1.22	>23
#2 (example 24)	0.03-1.22	>23
#3 (example 25)	0.002-0.05	>23
#4 (example 26)	0.02-0.05	>23
AZT (reference)	<0.002	>1

DETERMINATION OF IC50 (µM) IN DIFFERENT MUTATED ISOLATES OF HIV-1 IN THE PRESENCE OF DIFFERENT ANTIVIRAL COMPOUNDS

The procedure is similar to the procedure used in the CBM assay, with the exception that constructed virus and construted mutated virus were used.

The constructed virus HXB2D-65 correspond to the ddC resistance mutation and the constructed virus HXB2D-184 correspond to the 3TC™ and FTC resistance mutation.

TABLE 5 Values of IC^{50} in CBMCs with Differents Mutated Isolates of HIV-1

COMPOUNDS	III_B	HXB2D	HXB2D-65 K→R	HXB2D-184 M→V
#1	**0.22**	**1.0**	**2.5**	**0.53**
#2	**0.28**	**0.1**	**3.5**	**3.0**
#3	**0.83**	**3.0**	**11.5**	**9.0**
#4	**0.34**	**3.0**	**6.5**	**2.0**
AZT	**0.002**	**0.002**	**0.001**	**0.001**
3TC™	**0.01**	**0.08**	**0.125**	**42.5**

DETERMINATION OF IC50 (μM) IN HIV-1 ACUTELY INFECTED CELL LINES IN THE PRESENCE OF DIFFERENT ANTIVIRAL COMPOUNDS

Various cell lines were infected with HTLV-IIIb (TCID50 = 200) and then cultured with various concentrations of drug. IC50 was determined by measuring p24 antigen levels in the supernatant of cultures at day 6 of infection.

TABLE 6

COMPOUND	ID_{50} (μg/ml)				
	MT-4	Jurkat	H9	U937	CEM
#1 (example 23)	2.8	3.0	0.3	0.4	0.2
#2 (example 24)	0.9	1.8	0.075	0.3	0.4
#3 (example 25)	3.0	3.5	0.1	0.6	0.2
#4 (example 26)	3.2	6.0	1.0	0.4	0.3
AZT (reference)	0.005	0.01	0.07	0.04	0.01

EVALUATION OF CELL CULTURE INHIBITORY DOSE (CCID50)

Cell growth was determined by cell counting and viability by trypan blue exclusion 7 days post drug treatment. CCID50 is expressed as the drug concentration which inhibits 50% of cell growth.

TABLE 7 (CCID50 in Different Cells (μM))

COMPOUNDS	CBMCs	MT-4	U937	Jurkat	H9	CEM
#1 (example 23)	>500	>500	>500	450	>500	>500
#2 (example 24)	105	>500	22	11	>500	103
#3 (example 25)	>500	>500	>500	>500	>500	>500
#4 (example 26)	>500	>500	>500	>500	>500	>500
AZT	45	110	110	>500	200	500

INHIBITION OF HUMAN HEPATITIS B VIRUS.

The method used for this test is described in detail in Korba et al., Antiviral Research 15, 217-228 (1992) which is shortly described as follows:

Hep G2 cells transfected with human hepatitis B virus genomic DNA (2.2.15 cells) were grown and maintained in RPMI-1640 culture medium containing %5 foetal bovine serum, 2mM glutamine and 50μg/ml gentamicin sulphate, and checked routinely for G418 resistance. Cultures of 2.2.15 cells were grown to confluence in 24 well tissue culture plates and maintained for 2 to 3 days in that condition prior to drug treatment.

Drugs were dissolved in sterile water or sterile 50% DMSO in water at concentrations 100-fold higher than the higher test concentration. These solutions were diluted as needed in culture medium.

The culture medium on the confluent cells was changed 24 hours prior to exposure to test compounds. During the 10 day treatment, the culture medium was changed daily. After 10 days of the treatment, the culture medium was collected and frozen at -70°C for HBV DNA analysis.

To analyze extracellular HBV DNA, 0.2ml samples of culture medium were incubated for 20 minutes at 25°C in 1M NaOH/10X SSC (1X SSC is 0.15M NaCl/ 0.015M Sodium Citrate,

pH 7.2) and then applied to nitrocellulose membranes presoaked in 20X SSC. Filters were then rinsed in 2X SSC and baked at 80°C for 1 hour under vacuum.

A purified 3.2 kb EcoR1 HBV DNA fragment was labeled with [^{32}P]dCTP by nick translation and used as a probe to detect HBV DNA on the dot-blot by DNA hybridization. After washing, the hybridized blot was dried and ^{32}P was quantified using an Ambis beta scanner.

10

TABLE 8 (HBV)

COMPOUND	ID_{50} (µg/ml)	CD_{50} (µg/ml)
BCH -270 (example 7)	7.5	>10
Compound #2 (example 24)	4	>10

74872-53

CLAIMS:

1. Use of a pharmaceutical formulation for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections in mammals, the pharmaceutical formulation comprising:

2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

or a pharmaceutically acceptable salt or ester thereof in an amount effective for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections wherein 3TC™ is 2R-hydroxymethyl-5S-(cytosin-1'-yl)-1,3-oxathiolane and FTC is 2-hydroxymethyl-5-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier.

2. Use according to claim 1, for treating a human.

3. Use according to claim 1 or 2 wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

4. Use according to claim 1 or 2 wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-

oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

5. Use according to claim 1 or 2 wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

6. Use according to claim 1 or 2 wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

7. Use according to any one of claims 1 to 6 in a dose of 3-120 mg per kilogram of body weight per day.

8. Use according to any one of claims 1 to 6 in a dose of 6-90 mg per kilogram of body weight per day.

9. Use according to any one of claims 1 to 6 in a dose of 15-60 mg per kilogram of body weight per day.

10. Use according to any one of claims 1 to 9 for intravenous injection.

11. Use according to any one of claims 1 to 9 for oral administration.

12. Use according to any one of claims 1 to 11, wherein said pharmaceutically acceptable salts are selected from the group consisting of alkali metal salts, alkaline earth metal salts, ammonium salts, N-$(C_{1-4}$-alkyl$)_4^+$ salts and salts derived from hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, perchloric acid, fumaric acid, maleic acid, phosphoric acid, glycolic acid, lactic acid, salicylic acid, succinic acid, p-toluene sulfonic acid,

tartaric acid, acetic acid, citric acid, methanesulfonic acid, formic acid, benzoic acid, malonic acid, naphthalene-2-sulfonic acid, and benzenesulfonic acid, and

wherein said pharmaceutically acceptable esters are selected from the group consisting of methyl, ethyl, n-propyl, t-butyl, n-butyl, methoxymethyl, benzyl, phenoxymethyl, phenyl, N-methyldihydropyridinyl, methanesulfonyl, sulfate, L-valyl, L-isoleucyl, monophosphate, diphosphate and triphosphate esters.

13. Use according to claim 3, wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane.

14. Use according to claim 4, wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane.

15. Use according to claim 5, wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

16. Use according to claim 6, wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

17. Use according to any one of claims 1 to 16, wherein said pharmaceutical formulation contains 10-1500 mg per unit dosage of said compound.

18. Use according to claim 17, wherein said pharmaceutical formulation contains 20-1000 mg per unit dosage of said compound.

19. Use according to claim 18, wherein said pharmaceutical formulation contains 50 to 700 mg per unit dosage of said compound.

74872-53

20. Use according to claim 1, in a dose of 1-750 mg/kg/day.

21. Use according to any one of claims 1 to 20, wherein said pharmaceutical formulation additionally comprises a further therapeutic agent that is an anti-infective agent.

22. Use according to claim 21 wherein the further therapeutic agent is an antimicrobial agent.

23. Use according to claim 21 wherein the further therapeutic agent is an antiviral agent.

24. Use according to claim 21, wherein said further therapeutic agent is selected from the group consisting of AZT, 3TC™, ddC and ddI, wherein AZT is 3'-azido-3'-deoxythymidine, ddC is 2',3'-dideoxycytidine and ddI is 2',3'-dideoxyinosine.

25. Use according to claim 24, wherein said further therapeutic agent is 3TC™.

26. A pharmaceutical formulation for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections in mammals, the pharmaceutical formulation comprising:

2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

or a pharmaceutically acceptable salt or ester thereof in an amount effective for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier.

27. A formulation according to claim 26, for treating a human.

28. A formulation according to claim 26 or 27 wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

29. A formulation according to claim 26 or 27 wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

30. A formulation according to claim 26 or 27 wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

31. A formulation according to claim 26 or 27 wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

32. A formulation according to any one of claims 26 to 31 wherein a dose achieves a peak plasma concentration between 1 and 75 μM of said compound.

74872-53

33. A formulation according to any one of claims 26 to 31 in a dose of 3-120 mg per kilogram of body weight per day.

34. A formulation according to any one of claims 26 to 31 in a dose of 6-90 mg per kilogram of body weight per day.

35. A formulation according to any one of claims 26 to 31 in a dose of 15-60 mg per kilogram of body weight per day.

36. A formulation according to any one of claims 26 to 35 for intravenous injection.

37. A formulation according to any one of claims 26 to 35 for oral administration.

38. A formulation according to any one of claims 26 to 37, wherein said pharmaceutically acceptable salts are selected from the group consisting of alkali metal salts, alkaline earth metal salts, ammonium salts, N-$(C_{1-4}$-alkyl$)_4^+$ salts and salts derived from hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, perchloric acid, fumaric acid, maleic acid, phosphoric acid, glycolic acid, lactic acid, salicylic acid, succinic acid, p-toluene sulfonic acid, tartaric acid, acetic acid, citric acid, methanesulfonic acid, formic acid, benzoic acid, malonic acid, naphthalene-2-sulfonic acid, and benzenesulfonic acid, and

wherein said pharmaceutically acceptable esters are selected from the group consisting of methyl, ethyl, n-propyl, t-butyl, n-butyl, methoxymethyl, benzyl, phenoxymethyl, phenyl, N-methyldihydropyridinyl, methanesulfonyl, sulfate, L-valyl, L-isoleucyl, monophosphate, diphosphate and triphosphate esters.

74872-53

39. A formulation according to claim 28, wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane.

40. A formulation according to claim 29, wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane.

41. A formulation according to claim 30, wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

42. A formulation according to claim 31, wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

43. A formulation according to any one of claims 26 to 42, wherein said pharmaceutical formulation contains 10-1500 mg per unit dosage of said compound.

44. A formulation according to claim 43, wherein said pharmaceutical formulation contains 20-1000 mg per unit dosage of said compound.

45. A formulation according to claim 44, wherein said pharmaceutical formulation contains 50 to 700 mg per unit dosage of said compound.

46. A formulation according to claim 26, in a dose of 1-750 mg/kg/day.

47. A formulation according to any one of claims 26 to 46, wherein said pharmaceutical formulation additionally comprises a further therapeutic agent that is an anti-infective agent.

74872-53

48. A formulation according to claim 47 wherein the further therapeutic agent is an antimicrobial agent.

49. A formulation according to claim 47 wherein the further therapeutic agent is an antiviral agent.

50. A formulation according to claim 49, wherein said further therapeutic agent is selected from the group consisting of AZT, 3TC™, ddC and ddI.

51. A formulation according to claim 50, wherein said further therapeutic agent is 3TC™.

52. Use of:

2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

or a pharmaceutically acceptable salt or ester thereof in the preparation of a pharmaceutical formulation for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections, the pharmaceutical formulation further comprising a pharmaceutically acceptable carrier.

53. Use according to claim 52, wherein the pharmaceutical formulation is for treating a human.

74872-53

54. Use according to claim 52 or 53 wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

55. Use according to claim 52 or 53 wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

56. Use according to claim 52 or 53 wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

57. Use according to claim 52 or 53 wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

58. Use according to any one of claims 52 to 57 wherein a dose achieves a peak plasma concentration between 1 and 75 μM of said compound.

59. Use according to any one of claims 52 to 57 in a dose of 3-120 mg per kilogram of body weight per day.

60. Use according to any one of claims 52 to 57 in a dose of 6-90 mg per kilogram of body weight per day.

61. Use according to any one of claims 52 to 57 in a dose of 15-60 mg per kilogram of body weight per day.

62. Use according to any one of claims 52 to 61 for intravenous injection.

74872-53

63. Use according to any one of claims 52 to 61 for oral administration.

64. Use according to any one of claims 52 to 63, wherein said pharmaceutically acceptable salts are selected from the group consisting of alkali metal salts, alkaline earth metal salts, ammonium salts, N-(C_{1-4}-alkyl)$_4^+$ salts and salts derived from hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, perchloric acid, fumaric acid, maleic acid, phosphoric acid, glycolic acid, lactic acid, salicylic acid, succinic acid, p-toluene sulfonic acid, tartaric acid, acetic acid, citric acid, methanesulfonic acid, formic acid, benzoic acid, malonic acid, naphthalene-2-sulfonic acid, and benzenesulfonic acid, and

wherein said pharmaceutically acceptable esters are selected from the group consisting of methyl, ethyl, n-propyl, t-butyl, n-butyl, methoxymethyl, benzyl, phenoxymethyl, phenyl, N-methyldihydropyridinyl, methanesulfonyl, sulfate, L-valyl, L-isoleucyl, monophosphate, diphosphate and triphosphate esters.

65. Use according to claim 54, wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane.

66. Use according to claim 55, wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane.

67. Use according to claim 56, wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

68. Use according to claim 57, wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

74872-53

69. Use according to any one of claims 52 to 68, wherein said pharmaceutical formulation contains 10-1500 mg per unit dosage of said compound.

70. Use according to claim 69, wherein said pharmaceutical formulation contains 20-1000 mg per unit dosage of said compound.

71. Use according to claim 70, wherein said pharmaceutical formulation contains 50 to 700 mg per unit dosage of said compound.

72. Use according to claim 52, in a dose of 1-750 mg/kg/day.

73. Use according to any one of claims 52 to 72, wherein said pharmaceutical formulation additionally comprises a further therapeutic agent that is an anti-infective agent.

74. Use according to claim 73 wherein the further therapeutic agent is an antimicrobial agent.

75. Use according to claim 73 wherein the further therapeutic agent is an antiviral agent.

76. Use according to claim 73, wherein said further therapeutic agent is selected from the group consisting of AZT, 3TC™, ddC and ddI.

77. Use according to claim 76, wherein said further therapeutic agent is 3TC™.

78. A commercial package containing:

74872-53

2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane;

2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane; or any combination of the two isomers;

or a pharmaceutically acceptable salt or ester thereof, together with instructions for its use for treating 3TC™-resistant or FTC-resistant human immunodeficiency virus infections.

79. A package according to claim 78, for treating a human.

80. A package according to claim 78 or 79 wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

81. A package according to claim 78 or 79 wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

82. A package according to claim 78 or 79 wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

83. A package according to claim 78 or 79 wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-

1,3-oxathiolane, a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable ester thereof.

84. A package according to any one of claims 78 to 83 containing a dose of 3-120 mg per kilogram of body weight per day.

85. A package according to any one of claims 78 to 83 containing a dose of 6-90 mg per kilogram of body weight per day.

86. A package according to any one of claims 78 to 83 containing a dose of 15-60 mg per kilogram of body weight per day.

87. A package according to any one of claims 78 to 86 for intravenous injection.

88. A package according to any one of claims 78 to 86 for oral administration.

89. A package according to any one of claims 78 to 88, wherein said pharmaceutically acceptable salts are selected from the group consisting of alkali metal salts, alkaline earth metal salts, ammonium salts, N-$(C_{1-4}$-alkyl$)_4^+$ salts and salts derived from hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, perchloric acid, fumaric acid, maleic acid, phosphoric acid, glycolic acid, lactic acid, salicylic acid, succinic acid, p-toluene sulfonic acid, tartaric acid, acetic acid, citric acid, methanesulfonic acid, formic acid, benzoic acid, malonic acid, naphthalene-2-sulfonic acid, and benzenesulfonic acid, and

wherein said pharmaceutically acceptable esters are selected from the group consisting of methyl, ethyl, n-propyl, t-butyl, n-butyl, methoxymethyl, benzyl,

phenoxymethyl, phenyl, N-methyldihydropyridinyl, methanesulfonyl, sulfate, L-valyl, L-isoleucyl, monophosphate, diphosphate and triphosphate esters.

90. A package according to claim 80, wherein said compound is 2R-hydroxymethyl-4R-(cytosin-1'-yl)-1,3-oxathiolane.

91. A package according to claim 81, wherein said compound is 2S-hydroxymethyl-4S-(cytosin-1'-yl)-1,3-oxathiolane.

92. A package according to claim 82, wherein said compound is 2R-hydroxymethyl-4R-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

93. A package according to claim 83, wherein said compound is 2S-hydroxymethyl-4S-(5'-fluorocytosin-1'-yl)-1,3-oxathiolane.

94. A package according to any one of claims 78 to 93, which contains 10-1500 mg per unit dosage of said compound.

95. A package according to claim 94, which contains 20-1000 mg per unit dosage of said compound.

96. A package according to claim 95, which contains 50 to 700 mg per unit dosage of said compound.

97. A package according to claim 78, containing a dose of 1-750 mg/kg/day.

98. A package according to any one of claims 78 to 97, which additionally comprises a further therapeutic agent that is an anti-infective agent.

74872-53

99. A package according to claim 98, wherein said further therapeutic agent is selected from the group consisting of AZT, 3TC™, ddC and ddI, wherein AZT is 3'-azido-3'-deoxythymidine, ddC is 2',3'-dideoxycytidine and ddI is 2',3'-dideoxyinosine.

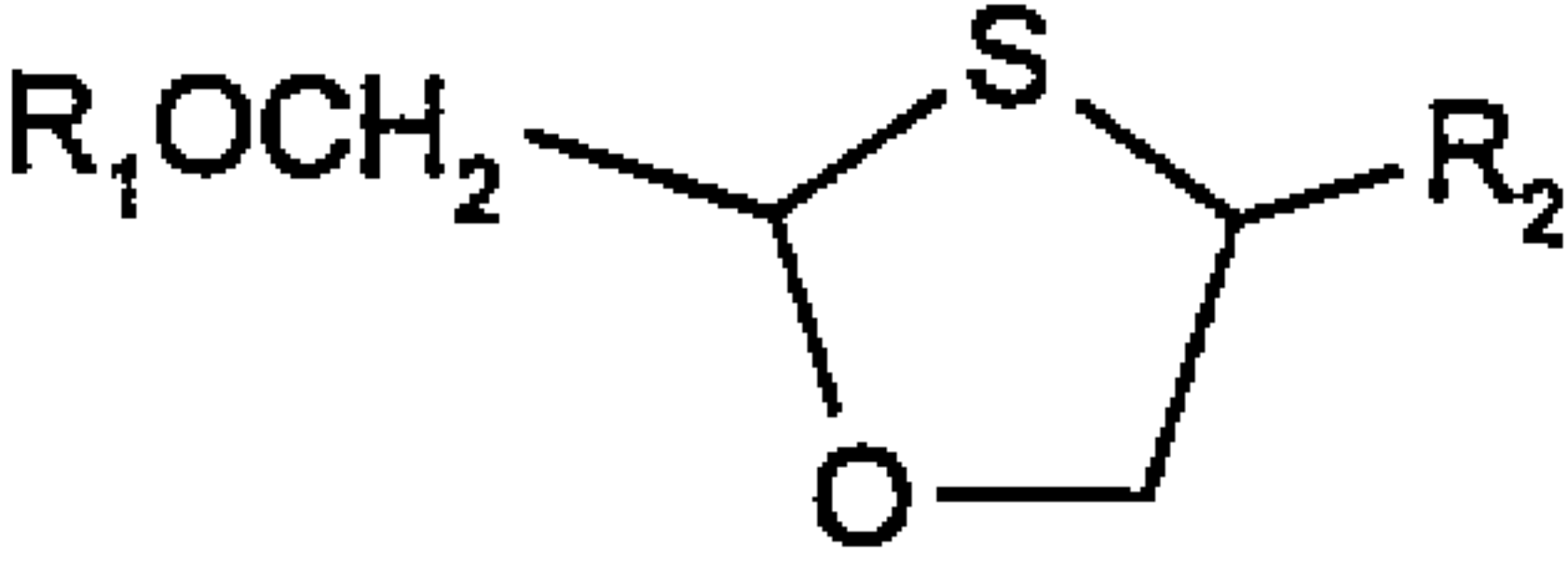
100. A package according to claim 99 wherein the further therapeutic agent is an antimicrobial agent.

101. A package according to claim 99 wherein the further therapeutic agent is an antiviral agent.

102. A package according to claim 101, wherein said further therapeutic agent is 3TC™.

SMART & BIGGAR
OTTAWA, CANADA

PATENT AGENTS



(I)