P/00/001 : Section 29

AUSTRALIA

Patents Act 1990

626278

PATENT REQUEST : STANDARD PATENT

We, **REGENTS OF UNIVERSITY OF MINNESOTA** being the person(s) identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification.

Full application details follow:

[71/70] Applicant/Nominated Person: REGENTS OF UNIVERSITY OF MINNESOTA

Address: 100 Church Street, SE, Minneapolis, Minnesota 55455
United States of America

[54] Invention Title: DIDEOXYDIDEHYDROCARBOCYCLIC NUCLEOSIDES

[72] Name(s) of actual inventor(s):

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Mei Hua
Peter Leslie Myers
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DAVIES COLLISON CAVE, Patent Attorneys, of Level 10, 10 Barrack Street, Sydney, New South Wales, Australia

AUSTRALIAN PATENT OFFICE

BASIC CONVENTION APPLICATION(S) DETAILS:

[31] Application No.	[33] Country	Code	[32] Date
146,252	USA	US	20 Jan 1988
8821011.7	UK	GB	7 Sept 1988
278,652	USA	US	5 Dec 1988

7 January, 1992

Hector Cumming
....
(a member of the firm of DAVIES COLLISON CAVE
for and on behalf of the Applicant).

P/00/008 Section 29(1)
Regulation 3.1(2)

A U S T R A L I A

Patents Act 1990

NOTICE OF ENTITLEMENT

We, REGENTS OF UNIVERSITY OF MINNESOTA, of 100 Church Street, SE, Minneapolis, Minnesota, 55455, United States of America, being the applicant and Nominated person in respect of Application No. 28671/89, state the following:-

The Nominated person(s) is entitled to the grant of the patent because the Nominated person(s) has entitlement from the actual inventor(s) by assignment and mesne assignment

The Nominated person(s) has entitlement from the applicant(s) of the basic application(s) listed on the patent request form by assignment and mesne assignment.

The basic application(s) listed on the patent request form are the first applications made in a Convention country in respect of the invention.

Dated this 17th day of February, 1992

A member of the firm of DAVIES COLLISON CAVE
for and on behalf of the applicant

(12) PATENT ABRIDGMENT (11) Document No. **AU-B-28671/89**
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. **626278**

(54) Title
DIDEOXYDIDEHYDROCARBOCYCLIC NUCLEOSIDES

International Patent Classification(s)

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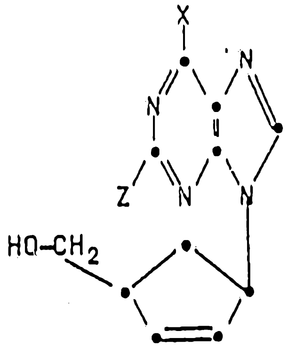
(56) Prior Art Documents
AU 37025/89 C07D 473/16

(57) Claim

1. A compound of formula (I)



(I)

wherein X is hydrogen, NRR^1, SR, OR or halogen;
Z is hydrogen, OR^2 or NRR^1;
R, R^1 and R^2 may be the same or different and are selected from hydrogen, C_{1-4} alkyl and aryl as herein before defined;
and pharmaceutically acceptable derivatives thereof as hereinbefore defined.

.../2

13. A method of treating viral infection in an animal including a human comprising administering to said animal an effective amount of a compound of formula (I) as defined in any one of claims 1 to 12.

AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE 626278

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority:
Related Art:

TO BE COMPLETED BY APPLICANT

Name of Applicant:	REGENTS OF THE UNIVERSITY OF MINNESOTA
Address of Applicant:	100 Church Street SE, Minneapolis, Minnesota 55455, U.S.A.
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Complete Specification for the invention entitled DIDEOXYDIDEHYDROCARBOCYCLIC NUCLEOSIDES.

The following statement is a full description of this invention including the best method of performing it known to me:-

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ASC 49

DIDEOXYDIDEHYDROCARBOCYCLIC NUCLEOSIDES

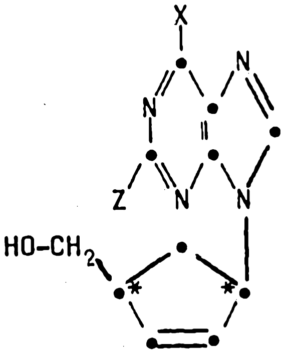
The present invention relates to dideoxycarbocyclic nucleoside analogues. More specifically it is concerned with carbocyclic 2',3'-dideoxy-2',3'-didehydro purine nucleoside analogues and their use in therapy, in particular as antiviral agents.

In view of the similarity between viral and host cellular functions it is difficult to selectively attack a virus while leaving the host cell intact. Thus, there are relatively few agents effective against viruses per se and it is difficult to find antiviral agents having an acceptable therapeutic index, i.e. agents which have a meaningful antiviral effect at a dose level at which the agent has an acceptable toxicity, or side effect, profile.

One group of viruses which have recently assumed major significance are the retroviruses responsible for the human acquired immunodeficiency syndrome (AIDS). Such viruses have previously been referred to by various terminologies but are now generally referred to as human immunodeficiency viruses (HIV's); two such viruses, HIV-I and HIV-II, have been reproducibly isolated from patients suffering from AIDS and related conditions such as AIDS related complex (ARC) and persistent generalised lymphadenopathy.

Although a number of nucleosides have been taught as useful in the treatment of conditions associated with HIV infections, only zidovudine (AZT, Retrovir) has received regulatory approval for the treatment of such conditions. However, it is known that zidovudine has severe side effects, causing suppression of the bone marrow leading to a drop in the white blood cell count with consequent pronounced anaemia, and there is a need for effective agents which are less cytotoxic.

We have now found a novel class of nucleoside analogues having antiviral activity. There is accordingly provided in a first aspect a compound of formula (I)



(I)

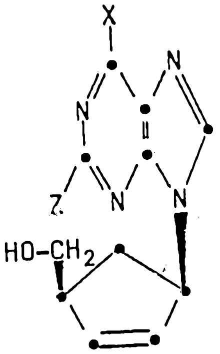
wherein X is hydrogen, NRR^1, SR, OR or halogen;

Z is hydrogen, OR^2 or NRR^1;

R, R^1 and R^2 may be the same or different and are selected from hydrogen, C_{1-4}alkyl and aryl;

and pharmaceutically acceptable derivatives thereof.

It will be appreciated by those skilled in the art that the compounds of formula (I) are cis compounds and further that the cyclopentene ring of the compounds of formula (I) contain two chiral centres (shown in formula (I) by *) and may thus exist in the form of two optical isomers (i.e. enantiomers) and mixtures thereof including racemic mixtures. All such isomers and mixtures thereof including racemic mixtures are included within the scope of the invention. Thus in the compounds of formula (I) either the chiral centre to which the base is attached is in the R configuration and the chiral centre to which the CH_2OH moiety is attached is in the S configuration (hereinafter the D isomer) or the chiral centre to which the base in attached is in the S configuration and that to which the CH_2OH moiety is attached is in the R configuration (hereinafter the L isomer). Conveniently the compounds will be in the form of either a racemic mixture or substantially as the pure D isomer. The D isomers may be represented by the formula (Ia)



(Ia)

where X and Z are as defined for formula (I). Reference hereinafter to compounds of formula (I) includes compounds of formula (Ia).

It will also be appreciated by those skilled in the art that cetain of the compounds of formula (I) may exist as a number of tautomeric forms and all such tautomers are included within the scope of the invention.

As used herein the term halogen refers to fluorine, chlorine, bromine and iodine; when X is halogen it is preferably chlorine.

As used herein C_{1-4} alkyl refers to a straight or branched chain alkyl group for example methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl and t-butyl. Conveniently C_{1-4} is methyl.

As used herein aryl refers to any unsubstituted mono- or polycyclic aromatic moiety and includes araklyl including ar (C_{1-4}) alkyl such as phen(C_{1-4})alkyl for example benzyl or phenethyl. In addition aryl refers to a phenyl group substituted by one or two methyl groups or a methoxy group.

In the compounds of formula (I) Z is preferably amino.

In one preferred class of compounds of formula (I) X is OR, in particular OH.

In a further preferred class of compounds of formula (I) X is NRR^1 in particular NH_2, or hydrogen.

Particularly preferred compounds of formula (I) are those wherein Z is NH_2 and X is H, NH_2 or, especially, OH. Such compounds in particular have especially desirable therapeutic indices as antiviral agents.

By "a pharmaceutically acceptable derivative" is meant any 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.~~

Preferred esters of the compounds of formula (I) include carboxylic acid esters in which the non-carbonyl moiety 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); sulphonate esters such as alkyl- or aralkylsulphonyl (e.g. methanesulphonyl); amino acid esters (e.g. L-valyl or L-isoleucyl) and mono-, di- or tri-phosphate esters.

With regard to the above described esters, unless otherwise specified, any alkyl moiety present advantageously contains 1 to 18 carbon atoms, particularly 1 to 4 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group.

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, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic 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 NR_4^+ (where R is C_{1-4}alkyl) salts.

References hereinafter to a compound according to the invention includes both compounds of formula (I) and their pharmaceutically acceptable derivatives.

Specific compounds of formula (I) include : -

$(1\alpha,4\alpha)$-4-(6-Chloro-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

(1α,4α)-4-(6-Hydroxy-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

(1α,4α)-4-(6-Amino-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

(1α,4α)-4-(6-Mercapto-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

(1α,4α)-4-(2-Amino-6-chloro-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

(1α,4α)-4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

(1α,4α)-4-(2,6-Diamino-9H-purin-9-yl)-2-cyclopentenyl-carbinol;

in the form of a racemic mixture or a single enantiomer.

The compounds of the invention either themselves possess antiviral activity and/or are metabolizable to such compounds. In particular these compounds are effective in inhibiting the replication of retroviruses, including human retroviruses such as human immunodeficiency viruses (HIV's), the causative agents of AIDS.

Certain compounds of the invention in particular those wherein Z is H also posssess anticancer activity.

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 retroviral infections, or an anticancer agent.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, in particular an infection caused by 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.

There is also provided in a further or alternative aspect 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 or use as an anticancer agent.

The compounds of the invention having antiviral activity are also useful in the treatment of AIDS related conditions such as AIDS-related complex (ARC), progressive generalised lymphadenopathy (PGL), AIDS-related neurological conditions (such as dementia or tropical paraparesis), anti-HIV antibody positive and HIV- positive conditions, Kaposi's sarcoma and thrombocytopenia purpura.

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

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

Certain of the compounds of formula (I) are also useful as intermediates in the preparation of other compounds of the invention.

It will be appreciated by those skilled in the art that reference 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 of from about 1 to about 750mg/kg e.g. from about 10 to about 750mg/kg of bodyweight per day, such as 3 to about 120mg 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 60mg/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 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg 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 about 75 μM, preferably about 2 to 50 μM, most preferably about 3 to about 30μM. 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 orally administered as a bolus containing about 1 to about 100mg 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, 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, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation 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, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, 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.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented 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 moulds.

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 in the form of drops.

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

For administration by inhalation the compounds according to the invention are conveniently delivered from an insufflator, nebuliser or a pressurised pack or other convenient means of delivering an aerosol spray. Pressurised packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurised 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 with other therapeutic agents for example other antiinfective 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.

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.

Suitable therapeutic agents for use in such combinations include acyclic nucleosides such as aciclovir, interferons such as α-interferon, renal excretion inhibitors such as probenicid, nucleoside transport inhibitors such as dipyridamole, 2',3'-dideoxynucleosides such as 2',3'-dideoxycytidine, 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine, 2',3'-dideoxythymidine and 2',3'-dideoxy-2',3'-didehydrothymidine and immunomodulators such as interleukin II (IL2) and granulocyte macrophage colony stimulating factor (GM-CSF), erythropoetin and ampligen.

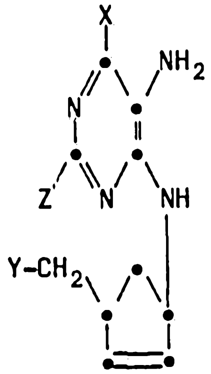
The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

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

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

Suitable methods for preparing compounds of formula (I) and their pharmaceutically acceptable derivatives are described below; the groups X and Z are as defined above except where otherwise indicated. It will be appreciated that the following reactions may require the use of, or conveniently may be applied to, starting materials having protected functional groups, and deprotection might thus be required as an intermediate or final step to yield the desired compound. Protection and deprotection of functional groups may be effected using conventional means. Thus, for example, amino groups may be protected by a group selected from 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 by hydrogenolysis in the presence of a noble metal catalyst such as palladium-on-charcoal. Silyl groups may also conveniently be removed using a source of fluoride ions such as tetra-n-butylammonium fluoride.

In a first process (A), compounds of formula (I) and pharmaceutically acceptable derivatives thereof may be prepared by reacting a compound of formula (II)



(II)

(wherein X and Z are substituents having the meaning in formula (I) or are protected forms thereof and Y is OH or a protected form thereof) or a pharmaceutically acceptable derivative thereof with a reagent selected from formic acid and reactive derivatives thereof, followed, where necessary, by removal of unwanted groups introduced by said reagent and/or by removal of any protecting groups present.

Examples of suitable derivatives of formic acid which may be used in process (A) above include orthoformates (e.g. triethyl orthoformate), dialkoxymethyl acetates (e.g. diethoxymethyl acetate), dithioformic acid, formamide, s-triazine or formamidine acetate.

Unwanted groups introduced by formic acid or a reactive derivative thereof may conveniently be removed by mild hydrolysis, for example using an inorganic acid such as aqueous hydrochloric acid.

When a trialkyl orthoformate such as triethyl orthoformate is used this is conveniently also the solvent for the reaction. Other solvents which may be used include amides (e.g. dimethylformamide or dimethylacetamide), chlorinated hydrocarbons (e.g. dichloromethane), ethers (e.g. tetrahydrofuran) or nitriles (e.g. acetonitrile).

In some cases (e.g. when a trialkyl orthoformate such as triethyl orthoformate is used) the reaction may preferably be carried out in the presence of a catalyst such as a strong acid (e.g. concentrated hydrochloric, nitric or sulphuric acid). The reaction may be effected at a temperature in the range of -25^0 to $+150\,^0C$, e.g. 0^0 to $100\,^0C$, and conveniently at ambient temperature.

In another process (B), compounds of formula (I) and their pharmaceutically acceptable derivatives or a protected form thereof are subjected to an interconversion reaction whereby the substituent X initially present is replaced by a different substituent X and/or the group Z initially present is replaced by a different group Z followed, where necessary by removal of any protecting groups present.

In one embodiment of process (B), compounds of formula (I) in which X represents a group NRR^1 (where R and R^1 are as defined previously) may be prepared by amination of a corresponding compound of formula (I) in which X represents a halogen atom (e.g. chlorine). The amination may be effected by reaction with a reagent $HNRR^1$ (where R and R^1 are as defined previously) conveniently in a solvent such as an alcohol (e.g. methanol). The reaction may be carried out at any suitable temperature and conveniently at an elevated temperature such as under reflux or, when liquid ammonia is used, in a sealed tube at about 50 to 80°C. Suitable conditions for the conversion of halides to secondary and tertiary amines have also been described by I. T. Harrison et. al., Compendium of Organic Synthetic Methods, Wiley-Interscience, New York (1971) at pages 250-252.

In another embodiment of process (B), compounds of formula (I) in which X represents a group OR (where R is as defined previously) may be prepared by displacement of the halogen (e.g. chlorine) atom with an appropriate anion RO^-. When R represents a hydrogen atom the displacement reaction may be carried out by hydrolysis which may be effected in water or in a mixture of water and a water-miscible solvent such as an alcohol (e.g. methanol or ethanol), an ether (e.g. dioxan or tetrahydrofuran), a ketone (e.g. acetone), an amide (e.g. dimethylformamide) or a sulphoxide (eg. dimethylsulphoxide), conveniently in the presence of an acid or base. Suitable acids include organic acids such as p-toluenesulphonic acid and inorganic acids such as hydrochloric, nitric or sulphuric acid. Suitable bases include inorganic bases such as alkali metal hydroxides or carbonates (e.g. sodium or potassium hydroxide or carbonate). Aqueous acid or base may also be used as the reaction solvent. The hydrolysis may conveniently be effected at a temperature in the range -10° to +150°C, e.g. at reflux. When R represents a C_{1-4}alkyl or aryl group the anion RO^- is conveniently formed from a corresponding alcohol ROH using an inorganic base such as an alkali metal (e.g. sodium metal) or an alkali metal hydride (e.g. sodium hydride). The reaction with the in situ formed anion may conveniently be effected at ambient temperature.

In a further embodiment of process (B), compounds of formula (I) in which X represents a group SH may be prepared by reacting the halo compound of formula (I) with thiourea in a suitable solvent such as an alcohol (e.g. n-propanol) at an elevated temperature (e.g. reflux) followed by alkaline hydrolysis. Suitable bases which may be used include alkali metal hydroxides (e.g. sodium hydroxide). The reaction may conveniently be carried out according to the method of G. G. Urquart et. al. Org. Syn. Coll. Vol. 3, 363(1953) eg by refluxing the intermediate product with aqueous NAOH for about 0.25 to about 5 hours.

In another embodiment of process (B), compounds of formula (I) in which X represents a hydrogen atom may be prepared by reducing the halo compound of formula (I) using a reducing system which will not affect the rest of the molecule. Suitable reducing agents which may be used to effect the desired dehalogenation reaction include zinc metal/water using the method described by J. R. Marshall et. al., J. Chem. Soc., 1004 (1951). Alternatively, the reaction may be effected by photolysis in a suitable solvent such as tetrahydrofuran containing 10% triethylamine and conveniently in a Rayonet photochemical reactor (2537A) according to the method of V. Nair et. al., J. Org. Chem., 52, 1344 (1987).

In a yet further embodiment of process (B), compounds of formula (I) in which X represents a halogen atom may be prepared from a different halo compound of formula (I) by conventional methods of halide-halide exchange. Alternatively, when X is chlorine this substituent may be replaced by other halogen atoms by using various p-(halo)benzene diazonium chlorides according to well-known procedures.

Compounds of formula (I) in which X represents a group SR where R is a C_{1-4}alkyl or aryl group may be prepared from the corresponding thiols using standard methods of alkylation or arylation for example as described in US Patent NO. 4,383,114.

Compounds of formula (I) in which Z represents a hydroxyl group may conveniently be prepared from a correspondig compound of formula (I) in which Z represents NH_2 by reaction with nitrous acid, for example employing the procedure used by J. Davoll in J. Amer. Chem. Soc., 73, 3174 (1951).

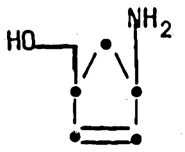
Many of the reactions described hereinabove have been extensively reported in the context of purine nucleoside synthesis, for example in Nucleoside Analogs - Chemistry, Biology and Medical Applications, R. T. Walker et. al., eds, Plenum Pless, New York (1979) at pages 193-223, the disclosure of which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

Pharmaceutically acceptable salts of the compounds of the invention may be prepared as described in US Patent No. 4,383,114, the disclosure of which is incorporated by reference herein. Thus, for example, when it is desired to prepare an acid addition salt of a compound of 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.

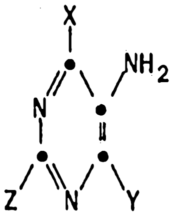
The compounds of formula (II) and salts thereof are novel compounds and form a further feature of the present invention.

The compounds of formula (II) in which Z represents hydrogen or hydroxyl may be prepared directly from the compound 2a



2a

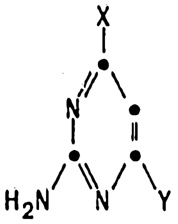
by reaction with an excess of a pyrimidine of formula (III)



(III)

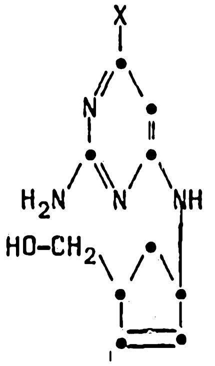
(wherein Y is a halogen atom, e.g. chlorine and Z is hydrogen or hydroxyl) in the presence of an amine base such as triethylamine and in an alcoholic solvent (e.g. n-butanol), conveniently at reflux.

Compounds of formula (II) in which Z represents NH_2 may be prepared using the compound of formula 2a by reaction with an excess of a pyrimidine of formula (IV)



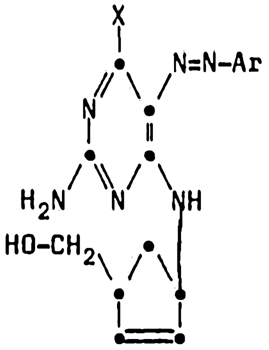
(IV)

(wherein Y is as defined in formula (III) above) under similar conditions to those described just above for the preparation of compounds of formula (II) in which Z represents hydrogen or hydroxyl to give a compound of formula (V)



(V)

which may be diazotized using a diazonium salt $ArN_2^+E^-$ (wherein Ar represents an aromatic group, e.g. p-chlorophenyl, and E^- represents an anion, e.g. a halide such as chloride) in a solvent such as water, an organic acid such as acetic acid or a mixture thereof, conveniently at about ambient temperature to give a compound of formula (VI)

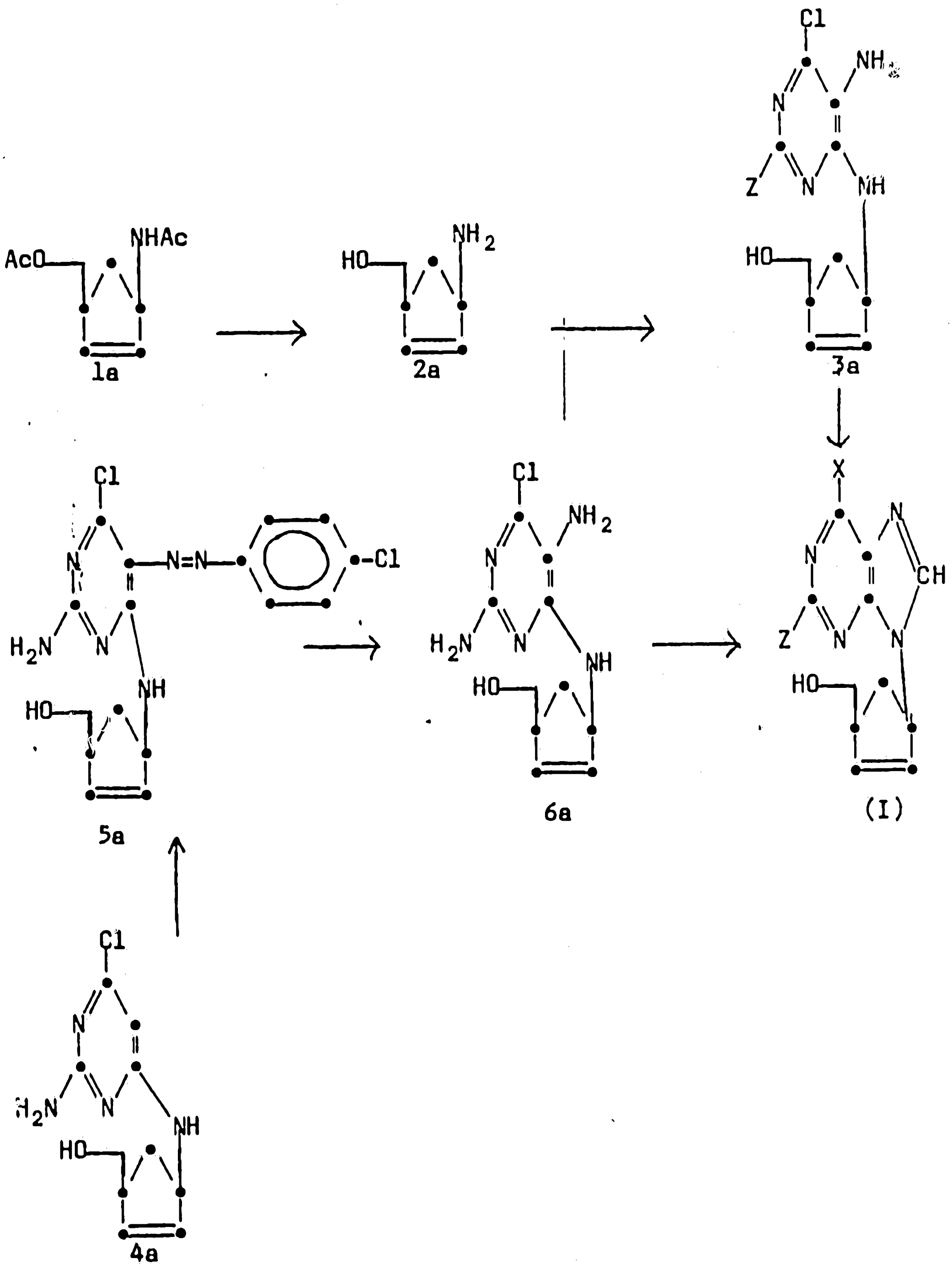


(VI)

(wherein Ar is as defined just above) which may be converted to the desired compound of formula (II) by reduction using for example a reducing metal such as zinc in the presence of an acid, e.g. acetic acid. It will be appreciated that the choice of reducing agent will depend on the nature of the group X.

The compound 2a may be prepared from the versatile precursor, 1α-acetylamino-3α-acetoxy-methylcyclopent-2-ene (1a) by hydrolysis in the presence of a mild base, such as an alkaline earth metal hydroxide.

A particularly convenient synthesis of compounds of formula (I) via 6-chloro compounds of formula (II) is outlined below.


AcO
NHAc
1a
HO
NH2
2a
Cl
NH2
N
Z
N
NH
HO
3a
X
N
N
CH
Z
N
N
HO
(I)
Cl
N
N=N
Cl
H2N
N
NH
HO
5a
Cl
NH2
N
H2N
N
NH
HO
6a
Cl
N
H2N
N
NH
HO
4a

The compound 2a and compounds of formulae (V) and (VI) are novel intermediates and form further features of the present invention.

The compound 1a is a known compound described in US Patent No. 4,138,562.

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

Resolution of the final product, or an intermediate or starting material therefor 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.

One convenient method for obtaining chirally pure compounds of formula (I) is by enzymatic conversion of a racemic mixture of the compound or a precursor thereof. By such a method both (+) and (-) compounds of formula (I) may be obtained in optically pure form. Suitable enzymes include deaminases such as adenosine deaminase.

The invention will be further described by reference to the following detailed examples wherein elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra were obtained on Jeol FX 90QFT or Nicollet NT300 spectrometers and were recorded in DMSO-D_6. Chemical shifts are expressed in ppm down-field from Me_4Si. IR spectra were determined as KBr pellets with a Nicollet 50XC FT-IR spectrometer, and UV spectra were determined on a Beckmann DU-8 spectrophotometer. Mass spectra were obtained with an AEI Scientific Apparatus Limited MS-30 mass spectrometer. Thin layer chromatography (TLC) was performed on 0.25mm layers of Merck silica gel (230-400 mesh). All chemicals and solvents are reagent grade unless otherwise specified. The term "active ingredient" as used in the Examples means a compound of formula (I) or a pharmaceutically acceptable derivative thereof.

Example 1

(±)-(1α,4α)-4-[(5-Amino-6-chloro-4-pyrimidinyl)-amino]-2-cyclopentenylcarbinol (3a)

A mixture of 1α-acetylamino-3α-acetoxymethyl cyclopent-2-ene (1a) (3.0g, 15 mmol) and aqueous barium hydroxide (0.5N, 300ml) was refluxed overnight. After cooling, it was neutralized with dry ice. The precipitate was filtered out, and the aqueous solution was concentrated to dryness. The residue was extracted with absolute ethanol and concentrated again to yield 2a as a colourless syrup 1.6g (14mmol).

To this syrup, 5-amino-4,6-dichloropyrimidine (4.59g 28 mmol), triethylamine (4.2g, 42 mmol), and n-butanol (50ml) were added and the mixture was refluxed for 24 hr. The volatile solvents were removed, the residue was absorbed on silica gel (7g), packed in a flash column (4.0 x 12cm) and eluted with $CHCl_3$-MeOH (20:1) to yield 2.69g (74%) of compound 3a; m.p. 130-132°C. An analytical sample was obtained by recrystallisation from ethyl acetate (EtOAc), m.p. 134-135°C, MS (30 ev, 200°C); m/e 240 and 242 (M^+ and M^++2), 209 (M^+ -31), 144 (B^+); IR: 3600-2600 (OH), 1620, 1580 (C=C, C=N); Anal. ($C_{10}H_{13}ClN_4O$) C, H, N.

Example 2

(±)-(1α,4α)-4-[(2-Amino-6-chloro-4-pyrimidinyl)-amino]-2-cyclopentenylcarbinol (4a)

To 14 mmol of crude 2a (Example 1) 2-amino-4,6-dichloropyrimidine (3.74g, 22.8 mmol), triethylamine (15ml) and n-butanol (75ml) were added and the mixture was refluxed for 48 hr. The volatile solvents were removed, residue was treated with methanol to separate the undissolved by- product (the double pyrimidine nucleoside). The methanol solution was absorbed on silica gel (8g) packed into a column (4.0 x 14cm) and eluted with $CHCl_3$-MeOH (40:1) to yield 1.52g (42%) of crude 4a. The product was recrystallised from ethyl acetate to yield 4a; m.p. 132-134°C, MS (30 ev, 200°C); m/e 240 and 242 (M^+ and M^++2), 209

(M^+-31), 144 (B^+); IR: 3600-3000 (NH2, OH), 1620,1580 (C=C, C=N); Anal. ($C_{10}H_{13}ClN_4$) C,H, N.

Example 3

(±)-(1α,4α)-4-{[(2-Amino-6-chloro-5-(4-chlorophenyl)-azo]-4-pyrimidinyl-amino}-2-cyclopentenylcarbinol (5a)

A cold diazonium salt solution was prepared from p-chloroaniline (1.47g, 11.5 mmol) in 3N HCl (25ml) and sodium nitrite (870mg, 12.5 mmol) in water (10ml). This solution was added to a mixture of 4a (2.40g, 10 mmol), acetic acid (50ml), water (50ml) and sodium acetate trihydrate (20g). The reaction mixture was stirred overnight at room temperature. The yellow precipitate was filtered and washed with cold water until neutral, then it was air-dried in the fumehood to yield 3.60g (94%), of 5a, m.p. 229°C (dec). The analytical sample was obtained from acetone-methanol (1:2), m.p. 241-243°C (dec). MS (30ev, 260°C): m/e 378 and 380 (M^+ and $M^+ + 2$), 282 (B^+); IR: 3600-3000 (NH_2, OH), 1620, 1580 (C=C, C=N); Anal. ($C_{16}H_{16}Cl_2N_6O$) C, H, N.

Example 4

(±)-(1α,4α)-4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)-amino]-2-cyclopentenylcarbinol (6a)

A mixture of 5a (379mg, 1 mmol), zinc dust (0.65g, 10 mmol), acetic acid (0.32 ml), water (15ml) and ethanol (15ml) was refluxed under nitrogen for 3 hr. The zinc was removed and the solvents were evaporated. The residue was absorbed on silica gel (2g), packed into a column (2.0 x 18cm), and eluted with $CHCl_3$-MeOH (15:1). A pink syrup was obtained. Further purification from methanol-ether yielded 6a as pink crystals, 170mg (66%), m.p. 168-170°C, MS (30 ev, 220°C); m/e 255 and 257 (M^+ and $M^+ + 2$), 224 ($M^+ -31$), 159 (B^+); IR: 3600-3000 (NH_2, OH) 1620,1580 (C=C, C=N); Anal. ($C_{10}H_{14}ClN_5$) C, H, N.

Example 5

(±)-(1α,4α)-4-(6-Chloro-9H-purin-9-yl)-2-cyclopentenyl-carbinol (7a)

A mixture of 3a (1.30g, 5.4 mmol), triethyl orthoformate (30ml) and hydrochloric acid (12N, 0.50ml) was stirred overnight at room temperature. The solvent was evaporated at 35°C *in vacuo*. To the residue was added aqueous hydrochloric acid (0.5 N, 30ml) and the mixture was stirred for 1hr., the mixture was neutralised to pH 7-8 with 1N sodium hydroxide and absorbed onto silica gel (8g), packed in a column (4.0 x 8cm), and eluted with $CHCl_3$-MeOH (20:1) to yield white crystals of 7a, 1.12g (82%). The crude product was recrystallised from ethyl acetate to yield 7a, m.p 108-110°C, MS (30 ev, 200°C); m/e 250 and 252 (M^+ and $M^+ + 2$), 219 ($M^+ - 31$), 154 (B^+); IR; 3600-2800 (OH), 1600 (C=C, C=N); Anal. ($C_{11}H_{11}ClN_4O$) C, H, N.

Example 6

(±)-(1α,4α)-4-(6-Hydroxy-9H-purin-9-yl)-2-cyclopentenyl-carbinol (8a)

A mixture of 7a (251mg, 1 mmol) and aqueous sodium hydroxide (0.2N, 10ml) was refluxed for 3hr. After cooling, the reaction mixture was adjusted to pH 5-6 with acetic acid. The reaction mixture was absorbed on silica gel (2g) packed in a column (2.0 x 11cm) and eluted with $CHCl_3$-MeOH (10:1) to yield 105mg (45%) of 8a. The crude white product was recrystallised from water-methanol (3:1) to yield 8a, m.p. 248-250°C (dec), Ms (30 ev, 300°C); m/e 232 (M^+), 214 ($M^+ - 18$), 136 (B^+); IR; 3600-2600 (OH), 1680,1600 (C=O, C=C, C=N); Anal. ($C_{11}H_{12}N_4O_2$) C, H, N.

Example 7

(±)-(1α,4α)-4-(6-Amino-9H-purin-9-yl)-2-cyclopentenyl-carbinol (9a)

Liquid ammonia was passed into a bomb containing a solution of 7a (250mg, 1 mmol) in methanol (5ml) at $^-80$° C. The bomb was sealed and heated at 60°C for 24hr. Ammonia and methanol were evaporated and the residue was recrystallised from water to yield off-white crystals of 9a, 187mg (81%), m.p. 198-200°C. MS (30 ev, 210°C): m/e 231 (M^+), 213 ($M^+ - 18$), 135 (B^+); IR: 3600-2600 (NH_2, OH), 1700,1600 (C=C, C=N); Anal. ($C_{11}H_{13}N_5O$) C, H, N.

Example 8

(±)-(1α,4α)-4-(6-Mercapto-9H-purin-9-yl)-2-cyclopentenyl-carbinol (10a)

A mixture of 7a (125mg, 0.5 mmol), thiourea (40mg, 0.64 mmol) and n-propanol (5ml) was refluxed for 2hr. After cooling, the precipitate was isolated by filtration, washed with n-propanol, and dissolved in sodium hydroxide (1N, 5ml). The solution was adjusted to pH 5 with acetic acid. The crude 10a (90mg, 73%) was isolated again, m.p.

260-262°C (dec) and was recrystallised from N,N-dimethylformamide, to yield 10a, m.p. 263-265°C (dec). MS (30 ev, 290°C): m/e 248 (M^+), 230 (M^+ $^-$18), 152 (B^+); IR: 3600-3200 (OH), 3100,2400 (SH), 1600 (C=C, C=N); Anal. ($C_{11}H_{12}N_4OS$) C, H, N.

Example 9

(±)-(1α,4α)-4-(2-Amino-6-chloro-9H-purin-9-yl)-2-cyclopentenyl-carbinol (13a)

A mixture of 6a (1.41g, 5.5 mmol) triethyl orthoformate (30ml) and hydrochloric acid (12N, 1.40ml) was stirred overnight. The suspension was dried in vacuo. Diluted hydrochloric acid (0.5N, 40ml) was added and the mixture was reacted at room temperature for 1hr. The mixture was neutralised to pH 8 with 1N sodium hydroxide and absorbed on silica gel (7.5g) packed in a column (4.0 x 10cm) and eluted by $CHCl_3$-MeOH (20:1) to yield off-white crystals of 13a, 1.18g (80%). The crude product was recrystallised from ethanol to yield 13a, m.p. 145-147°C. MS (30 ev, 220°C): m/e 265 and 267 (M^+ and M^++2), 235 (M^+ $^-$30), 169 (B^+); IR: 3600-2600 (NH_2, OH), 1620-1580 (C=C, C=N); Anal. ($C_{11}H_{12}N_5OCl.3/4\ H_2O$) C, H, N.

Example 10

(±)-(1α,4α)-4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2-cyclopentenyl carbinol (14a)

A mixture of 13a (266mg, 1 mmol) and aqueous sodium hydroxide (0.33N) was refluxed for 5hr, absorbed onto silica gel (2g) packed in a column (2.0 x 7.5cm) and eluted with $CHCl_3$-MeOH (5:1). The crude product was recrystallised from methanol-water (1:4) to yield white crystals of 14a, 152mg (61%), m.p. 254-256°C (dec). MS (30 ev, 200°C): m/e 247 (M^+), 217 (M^+ $^-$30), 151 (B^+); IR: 3600-2600 (NH_2, OH), 1700,1600 (C=O, C=C, C=N); Anal. ($C_{11}H_{13}N_5O_2.3/4\ H_2O$) C, H, N.

Example 11

(±)-(1α,4α)-4-(2,6-Diamino-9H-purin-9-yl)-2-cyclopentenyl carbinol (15a)

Liquid ammonia was passed into a solution of 13a (265mg, 1 mmol) in methanol (10ml) at $^-$80°C in a bomb. The bomb was sealed and heated at 75°C for 48hr. Ammonia and methanol were evaporated. The residue was absorbed on silica gel (2g), packed in a column (2.0 x 10cm) and eluted with $CHCl_3$-MeOH (15:1). The crude product was recrystallised from ethanol to yield 196mg (80%) of 15a, m.p. 152-155°C. MS (30 ev, 200°C): m/e 246 (M^+), 229 (M^+ -17), 216 (M^+ -30), 150 (B^+); IR: 3600-3000 (NH_2, OH), 1700,1650,1600 (C=O, C=C, C=N); Anal. ($C_{11}H_{14}N_6O$) C, H, N.

Example 12

(1S,4R)-4-(2,6-Diamino-9H-purin-9-yl)-2-cyclopentenyl carbinol

[(1S,4R)-4-(2,6-Diamino-9H-purin-9-yl)-2-cyclopentene methanol]

(a) Intermediate 1 : (1R,2S,3R,5R)-3-[6-Amino-9H-purin-9-yl]-5-[((1,1-dimethylethyl)-dimethylsilyloxy)methyl]-1,2-cyclopentanediol

(-) Aristeromycin[1] (12.505g), *tert*-butyldimethylsilyl chloride (7.8g) and imidazole (12.96g) in dry dimethylformamide (85ml) was stirred at ambient temperature for 2½ hours. The resulting solution was diluted with ethyl acetate (500ml), then washed with water (3x100ml) and brine (50ml) before a white solid crystallised out. This was collected by filtration, washed with ethyl acetate, then dried *in vacuo* to give the title product (3.92g); ^1H n.m.r. (DMSO-d_6) 8.15 (1H), 8.09 (1H), 7.19 (2H), 5.00 (1H), 4.72 (1H), 4.69 (1H), 4.36 (1H), 3.85 (1H), 3.67 (2H), 2.23 (1H), 2.09 (1H), 1.79 (1H), 0.89 (9H), 0.07 (6H).

1. Journal of the American Chemical Society 1983, vol. 105, 4049-4055.

(b) Intermediate 2 : (4R,3aS,6R,6aR)-4-[6-Amino-9H-purin-9-yl]-6-[((1,1-dimethylethyl)-dimethylsilyloxy)methyl]-3a,5,6,6a-tetrahydro-4H-cyclopenta-1,3- dioxole-2-thione

A stirred suspension of Intermediate 1 (3.45g) in dry dimethylformamide (56ml) was treated with 1,1'-thiocarbonyldiimidazole (3.3g), giving a yellow solution. After 15½ hours at ambient temperature the resulting solution was combined with that from a previous experiment (6% scale), and solvent was removed by evaporation. The residual oil was diluted with ethyl acetate (100ml), then washed with water (2x20ml) and brine (2x20ml), dried ($MgSO_4$) and evaporated to a yellow solid. This was washed with diethyl ether (25ml), then collected by filtration, further washed with ether (25ml), then dried in vacuo to give the title product as a pale cream solid (3.61g); λ_{max} (ethanol) 240.0nm ($E^{1\%}_{1cm}$ 459); ^{1}H n.m.r. (DMSO-d_6) 8.27 (1H), 8.13 (1H), 7.33 (2H), 5.81 (1H), 5.37 (1H), 5.28 (1H), 3.78 (2H), 2.60 (1H), 2.28 (2H), 0.90 (9H), 0.09 (6H).

(c) Intermediate 3 : (1'R,4'S)-9-[4-(((1,1-Dimethylethyl) dimethylsilyloxy)methyl)-2-cyclopenten-1-yl]-9H-purin-6-amine

A solution of Intermediate 2 (3.57g) in dry tetrahydrofuran (25ml) was treated with a solution of 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine (4.94g) in dry tetrahydrofuran (10ml), then stirred at ambient temperature for 8¾ hours. The solvent was removed by evaporation. The residual oil was combined with that from a previous experiment (40% scale), then subjected to column chromatography on silica (200g, Merck 7734), eluted with chloroform, then chloroform-ethanol mixtures to give a white solid. This solid was washed with diethyl ether (25ml), then collected by filtration. The solid was further washed with ether (10ml), then dried in vacuo to give the title product (1.47g); λ_{max} (ethanol) 261.4nm ($E^{1\%}_{1cm}$ 443); 1H n.m.r. (DMSO-d_6) 8.14 (1H), 8.00 (1H), 7.20 (2H), 6.12 (1H), 5.95 (1H), 5.60 (1H), 3.66 (2H), 2.96 (1H), 2.69 (1H), 1.65 (1H), 0.74 (9H), 0.02 (6H).

(d) Intermediate 4 : (1'R,4'S)-9-[4-(((1,1-Dimethylethyl) dimethylsilyloxy)methyl)-2-cyclopenten-1-yl]-9H-purin-6-amine,1-oxide

A solution of Intermediate 3 (1.37g) in chloroform (30ml) was treated with 80-90% m-chloroperoxybenzoic acid (1.29g), then stirred at ambient temperature for 3 hours. Solvent was removed by evaporation and the residual gum was dissolved in ethyl acetate (10ml). A white solid crystallised out. This solid and material recovered by evaporation of the filtrate were dissolved in chloroform (100ml), then washed with saturated aqueous sodium bicarbonate solution (3x10ml) and brine (2x10ml). The aqueous washings were back-extracted with chloroform (50ml). The combined organic solutions were dried ($MgSO_4$), then evaporated to a solid. This solid was washed with diethyl ether (25ml), then collected by filtration. The white solid was further washed with ether (10ml), then dried in vacuo to give the title product (1.16g); λ_{max} (ethanol) 235.4nm ($E^{1\%}_{1cm}$ 1324), 263.2nm ($E^{1\%}_{1cm}$ 248), 300.2nm ($E^{1\%}_{1cm}$ 75); ^1H n.m.r. ($CDCl_3$) 8.72 (1H), 8.02 (1H), 7.16 (2H), 6.21 (1H), 5.87 (1H), 5.72 (1H), 3.68 (2H), 3.04 (1H), 2.82 (1H), 1.74 (1H), 0.89 (9H), 0.06 (6H).

(e) Intermediate 5 : (1'R,4'S)-7-[4-(((1,1-Dimethylethyl) dimethylsilyloxy)methyl)-2-cyclopenten-1-yl]-2-imino-1,2-dihydro [1,2,4]oxadiazolo[3,2-i]-9H-purine hydrobromide

A stirred, ice-chilled suspension of Intermediate 4 (1.08g) in methanol (20ml) was treated with a solution of cyanogen bromide (0.34g) in methanol (20ml) added over 5 minutes. After 15 minutes, the suspension was allowed to warm to ambient temperature, giving a solution. After 90 minutes, solvent was removed by evaporation. The residue was washed with diethyl ether (25ml), then collected by filtration. The solid was further washed with ether (25ml), then dried in vacuo to give the title product (1.37g); λ_{max} (ethanol) 228.2nm ($E^{1\%}_{1cm}$ 530), 285.2nm ($E^{1\%}_{1cm}$ 445); ^1H n.m.r. ($CDCl_3$) 10.20 (1H), 10.02 (1H), 8.37 (1H), 6.25 (1H), 6.01 (1H), 5.90 (1H), 3.69 (2H), 3.05 (1H), 2.86 (1H), 1.73 (1H), 0.86 (9H), 0.03 (6H).

(f) Intermediate 6 : (1'R,4'S)-9-[4-(((1,1-Dimethylethyl) dimethylsilyloxy)methyl)-2-cyclopenten-1-yl]-6-cyanoimino-1,6-dihydro-1-methoxy-9H-purine

A solution of Intermediate 5 (1.36g) in dimethylformamide (10ml) was stirred at ambient temperature, then treated with triethylamine (1.2ml). After 40 minutes iodomethane (0.54ml) was added, giving a yellow solution. After 3¾ hours solvent was removed by evaporation. The residue was partitioned between ethyl acetate (100ml) and water (20ml). The organic solution was further washed with water (2x20ml) and brine (20ml), dried ($MgSO_4$) and evaporated to a solid. This solid was washed with diethyl ether (25ml), then collected by filtration. This white solid was further washed with ether (10ml), then dried in vacuo to give the title product (0.865g); λ_{max} (ethanol) 227.2nm ($E^{1\%}_{1cm}$ 449), 287.0nm ($E^{1\%}_{1cm}$ 544); 1H n.m.r. 8.23 (1H), 7.96 (1H), 6.24 (1H), 5.85 (1H), 5.65 (1H), 4.21 (3H), 3.66 (2H), 3.04 (1H), 2.77 (1H), 1.68 (1H), 0.88 (9H), 0.05 (6H).

(g) Intermediate 7 : (1'R,4'S)-9-[4-(((1,1-Dimethylethyl) dimethylsilyloxy)methyl)-2-cyclopenten-1-yl]-6-methoxyamino-9H-purin-2-amine

A solution of Intermediate 6 (802mg) and 1,8-diazabicyclo[5,4,0]undec-7-ene (0.45ml) in ethanol (80ml) was stirred and heated at reflux. Heating was stopped after 9 hours, and the solution was left at ambient temperature overnight. Solvent was removed by evaporation. The residual oil was combined with that from a previous experiment (4% scale), then subjected to column chromatography on silica (40g, Merck 9385) eluted with chloroform, then chloroform-ethanol mixtures to give a foam. This foam was triturated with diethyl ether (10ml) and the resulting solid was collected by filtration. The solid was further washed with ether (5ml), then dried in vacuo to give the title product (594mg); λ_{max} (ethanol) 282.2nm ($E^{1\%}_{1cm}$ 409); ^{1}H-n.m.r. (DMSO-d_6) 9.76 (1H), 7.32 (1H), 6.53 (2H), 6.08 (1H), 5.88 (1H), 5.26 (1H), 3.72 (3H), 3.61 (2H), 2.90 (1H), 2.50 (1H), 1.52 (1H), 0.83 (9H), 0.02 (6H).

(h) Intermediate 8 : (1S,4R)-4-[2-Amino-6-methoxyamino-9H-purin-9-yl]-2-cyclopentene-methanol

A solution of Intermediate 7 (356mg) in tetrahydrofuran (35ml) was stirred at ambient temperature then treated with tetrabutylammonium fluoride (1.0M solution in tetrahydrofuran, 1.4ml). After 90 minutes the reaction was quenched with water (1ml), then solvents were removed by evaporation. The residual oil was subjected to column chromatography on silica (20g, Merck 7734), eluted with chloroform, then chloroform-ethanol mixtures to give the title product as a solid (243mg); λ_{max} (pH 6 buffer) 280.2nm ($E^{1\%}_{1cm}$ 534); ^{1}H n.m.r. (DMSO-d_6)

9.75 (1H), 7.39 (1H), 6.52 (2H), 6.10 (1H), 5.84 (1H), 5.27 (1H), 4.73 (1H), 3.40 (2H), 2.83 (1H), 2.55 (1H), 1.52 (1H).

(1S,4R)-4-[2,6-Diamino-9H-purin-9-yl]-2-cyclopentenecarbinol

A stirred, ice-chilled solution of Intermediate 8 (210mg) in water (10ml) and tetrahydrofuran (50ml) was treated with aluminium amalgam [from aluminium (237mg) and 0.5% aqueous mercuric chloride solution], added in small pieces over 15 minutes. After 40 minutes the stirred mixture was allowed to warm to ambient temperature. After 15 hours the resulting mixture was filtered through kieselguhr to remove insolubles. These were washed with water:tetrahydrofuran (1:5, 60ml). The combined filtrates were evaporated. The residue was subjected to column chromatography on silica (10g, Merck 9385), eluted with chloroform-ethanol mixtures to give the title product as a foam (159mg); $[\alpha]_D$ -81^0 (c1.04, methanol); λ_{max} (pH 6 buffer) 255.0 nm ($E^{1\%}_{1cm}$ 302), 280.8 nm ($E^{1\%}_{1cm}$ 381), ^{1}H n.m.r. (DMSO-d_6) 7.61 (1H), 6.66 (2H), 6.10 (1H), 5.87 (1H), 5.76 (2H), 5.38 (1H), 4.76 (1H), 3.45 (2H), 2.87 (1H), 2.60 (1H), 1.60 (1H).

Example 13

(1S,4R)-4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2-cyclopentenyl carbinol

(1'R,4'S)-2-Amino-1,9-dihydro-9-[4-hydroxymethyl-2-cyclopenten-1-yl]-6H-purin-6-one

A turbid solution of the title compound of Example 12 (144mg) in 0.1M pH 6 buffer (10ml) (from 28.4g disodium orthophosphate in 2 litres of water, adjusted with orthophosphoric acid) was treated with a solution of adenosine deaminase (0.5ml, 778 units), in 50% glycerol - 0.01M potassium phosphate, pH 6.0, then stirred and warmed to 37^0. After 18½ hours the resulting suspension was refrigerated. The collected solid was recrystallised from water to give the title product as a white solid (86mg); $[\alpha]_D$$-49^0$ (c 0.5, dimethylsulphoxide); λ_{max} (pH 6 buffer) 252.6nm ($E^{1\%}_{1cm}$ 531), ^{1}H n.m.r. (DMSO-d_3) 10.60 (1H), 7.60 (1H), 6.47 (2H), 6.10 (1H), 5.86 (1H), 5.33 (1H), 4.72 (1H), 3.45 (2H), 2.59 (1H), 1.58 (1H).

Example 14

Preparation of Enantiomers of $(1\alpha,4\alpha)$-4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2-cyclopentenylcarbinol

(a) (1S, 4R)-4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2- cyclopentenyl carbinol

The diamino analog (100mg) (Example 11) was dissolved in 3ml of 0.05M K_2PO_4 buffer (pH 7.4) with heat (50° C). The solution was cooled to room temperature and 40 units of adenosine deaminase (Sigma, Type VI, calf intestinal mucosa) was added. After three days of incubation at room temperature a precipitate formed and was removed by filtration, yield, 18.2mg. The filtrate was concentrated to 1.5ml and refrigerated for 2 days. Additional solid was obtained by filtration, yield, 26.8mg. The two solid fractions were recrystallized from water and gave the pure title product m.p. 269-272°C, $[\alpha]_D^{24}$ - 62.1 (c 0.3 MeOH).

(b) (1R, 4S)-4-(2-Amino-6-hydroxy-9H-purin-9yl)-2- cyclopentenyl carbinol

The filtrates from the preparation of the 1S, 4R isomer (Example 14a) were combined and evaporated to dryness. The unchanged diamino starting material was separated on a silica gel flash column using 10% methanol/chloroform. The diamino compound was dissolved in 0.05M K_2PO_4 buffer, pH 7.4 (15ml) and 800 units of adenosine deaminase were added. The solution was incubated for 96 hours at 37°C. TLC indicated some unreacted product remained. The solution was heated in boiling water for 3 minutes and filtered to remove denatured protein. Another 800 units of adenosine deaminase were added and the process was repeated. The deproteinated solution was evaporated to dryness and

the product was crystallized from water. The title product as a white solid was collected by filtration from water, m.p. 265-270°. $[\alpha]_D^{24}$ + 61.1 (c0.3 MeOH).

Example 15

(±) (1α,4α)-4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2-cyclopentenyl acetoxycarbinol

To a suspension of the product of Example 10 (130 mg, 0.50 mmol) and 4-dimethylaminopyridine (5mg, 0.04mmol) in a mixture of acetonitrile (6ml) and triethylamine (0.09ml, 0.66mmol) was added acetic anhydride (0.06ml, 0.6 mmole). The mixture was stirred at room temperature for 3 hrs. Methanol (1ml) was added to quench the reaction. The solution was concentrated and absorbed on silica gel (1.5g), packed on a column (2.0 x 12cm), eluted with $CHCl_3$-MeOH (20:1). The product fractions were collected and concentrated to get white solid. The solid product was washed with MeOH-AcOEt: yield, 123mg (85%). Further purification from methanol gave the title product as needle-like crystals, m.p. 237-239°C. Anal. ($C_{13}H_{15}N_5O_3$)C,H,N.

Example 16

(1S,4R)-4-[2-amino-9H-purin-9-yl]-2-cyclopentenylcarbinol

A stirred, ice-chilled solution of (1S,4R)-4-[2-amino-6-methoxyamino-9H-purin-9-yl]-2-cyclopentene-methanol (intermediate 8, Example 12) (1.202g) in tetrahydrofuran (250ml) and water (50ml) was treated with aluminium amalgam (from aluminium (1.761g) and 0.5% aqueous mercuric chloride solution), added in small pieces over 1hr 47min. After 35 min the stirred mixture was allowed to warm to ambient temperature. After 16hr 50min more aluminium amalgam (from 235mg aluminium) was added over 14min. After a further 4hr 10min the resulting mixture was filtered through kieselguhr to remove insolubles. These were washed with tetrahydrofuran:water (5:1, 300ml). The combined filtrates were evaporated to leave a yellow foam. The foam was subjected to column chromatography on silica (33.8g, Merck 7734) prepared in chloroform and eluted with chloroform-ethanol mixtures to give several fractions (578mg, 420mg and 40mg). The two larger fractions were separately crystallised from iso-propanol. The filtrates were combined with the smallest column fraction and subjected to preparative thin layer chromatography (Merck 5717) developed three times in 10:1 chloroform:methanol. The plates were eluted with ethyl acetate and ethyl acetate-ethanol (1:1) to give a brown solid (45mg). The solid was subjected to column chromatography on silica (2.7g, Merck 7734) prepared in chloroform and eluted with chloroform-methanol-triethylamine mixtures to give a gum (17mg). Following an unsuccessful crystallisation from iso-propanol and charcoal treatment in methanol, an aqueous solution of the recovered material was freeze dried to give the title compound (15mg). ^{1}Hnmr (DMSO-d_6) 1.62 (1H), 2.63 (1H), 2.89 (1H), 3.45 (2H), 4.73 (1H), 5.48 (1H), 5.91 (1H), 6.14 (1H), 6.50 (2H), 7.98 (1H), 8.57 (1H). Mass spec, $[MH]^+$ 232.

Example 17

Tablet Formulations

A. The following formulation is prepared by wet granulation of the ingredients with a solution of povidone in water, drying and screening, followed by addition of magnesium stearate and compression.

		mg/tablet
(a)	Active ingredient	250
(b)	Lactose B.P.	210
(c)	Povidone B.P.	15
(d)	Sodium Starch Glycollate	20
(e)	Magnesium Stearate	5
		500

B. The following formulation is prepared by direct compression; the lactose is of the direct compression type.

	mg/tablet
Active ingredient	250
Lactose	145
Avicel	100
Magnesium Stearate	5
	500

C. (Controlled Release Formulation) The formulation is prepared by wet granulation of the ingredients (below) with a solution of povidone in water, drying and screening followed by the addition of magnesium stearate and compression.

		mg/tablet
(a)	Active ingredient	500
(b)	Hydroxypropylmethylcellulose (Methocel K4M Premium)	112
(c)	Lactose B.P.	53
(d)	Povidone B.P.	28
(e)	Magnesium Stearate	7
		700

Example 18

Capsule Formulation

A capsule formulation is prepared by admixing the ingredients below and filling into a two-part hard gelatin capsule.

	mg/capsule
Active ingredient	125
Lactose	72.5
Avicel	50
Magnesium Stearate	2.5
	250

Example 19

Injectable Formulation

Active ingredient 0.200g
Sodium hydroxide solution, 0.1M q.s. to a pH of about 11.
Sterile water q.s. to 10ml

The active ingredient is suspended in some of the water (which may be warmed) and the pH adjusted to about 11 with a solution of sodium hydroxide. The batch is then made up to volume and filtered through a sterilising grade membrane filter into a sterile 10ml glass vial and sealed with sterile closures and overseals.

Example 20

Suppository

	mg/suppository
Active ingredient (63μm)	250
Hard Fat, BP	1770
	2020

One-fifth of the hard fat is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200μm sieve and added to the molten base with mixing, using a high shear stirrer, until a smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining hard fat is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250μm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C, 2.02g of the mixture is filled into suitable, 2ml plastic moulds. The suppositories are allowed to cool to room temperature.

Example 21 - ANTIVIRAL ACTIVITY

(A) Anti-HIV Assay

Compounds of formula (I) were screened for anti-HIV activity at the National Cancer Institute, Frederick Cancer Research Facility, Frederick, Maryland (FCRF). The following are the current screening mode operational procedures utilized at FCRF. The protocol consists of 3 areas, (I) preparation of infected cells and distribution to the test plates, (II) preparation of drug dilution plates and distrubition to the test plates, and (III) XTT assay procedure. See D. A. Scudiero et al., "A New Simplified Tetrazolium Assay for Cell Growth and Drug Sensitivity in Culture," Cancer Res., 48, 4827 (1988).

I. Infection and Distribution of ATH8 Cells to Microtiter Trays

Cells to be infected (a normal lymphoblastoid cell line which expresses CD4) are placed in 50ml conical centrifuge tubes and treated for 1 hr with 1-2μg/ml of polybrene at 37°C. The cells are then pelleted for 8min. at 1200 RPM. HIV virus, diluted 1:10 in media (RMP1-1640, 10% human serum or 15% fetal calf serum (FCS), with IL-2 and antibiotics) is added to provide an MOI of .001. Medium alone is added to virus-free control cells. Assuming an infectious virus titer of 10^{-4}, an MOI of .001 represents 8 infectious virus particles per 10,000 cells. About 500,000 cells/tube are exposed to 400μl of the virus dilution. The resultant mixture is incubated for 1hr at 37°C in Air-CO_2. The infected or uninfected cells are diluted to give 1 x 10^{-4} (with human serum or 2 x 10^{-4} (with fetal calf serum) cells/100μl.

Infected or uninfected cells (100μl) are distributed to appropriate wells of a 96 well, U-bottom microtiter plate. Each compound dilution is tested in duplicate with infected cells. Uninfected cells are examined for drug sensitivity in a single well for each dilution of compound. Drug-free control cells, infected and uninfected, are run in triplicate. Wells B2 through G2 served as reagent controls and received medium only. The plates are incubated at 37^{0}C in Air-CO$_2$ until the drug is added.

II. Drug Dilution and Addition

Dilution plates (flat bottom 96 well, microtiter plates) are treated overnight with phosphate buffered saline (PBS) or media containing at least 1% FCS or 1% human serum (depending on the medium used in the test), beginning the day before assay. This "blocking" procedure is used to limit the adsorption of drug to the microtiter tray during the dilution process. The wells are filled completely with the blocking solution and allowed to stand at room temperature in a humidified chamber in a hood.

The dilution process is begun by first diluting the test compound 1:20. Blocked, dilution plates are prepared by flicking out the blocking solution and blotting dry on sterile gauze. All wells of each plate are then filled with 225µl of the appropriate medium using a Cetus liquid handling system. Twenty-five microliters (25µl) of each 1:20 diluted compound is then manually added to row A of a blocked and filled dilution plate. Four compounds, sufficient to supply two test plates, are added per dilution plate. The four compounds are then serially diluted 10 fold from row A through row H using the Cetus liquid handling system. The starting dilution of each compound in row A is, at this point, 1:200. The dilution plates are kept on ice until needed.

Using a multi-channel pipettor with 6 microtips, 100µl of each drug dilution is transferred to the test plate which already contains 100µl of medium plus cells. The final dilution, in the test plate, starts at 1:400 (wells B4 through G4). This dilution (to .25% DMSO) prevents the DMSO vehicle from interfering with cell growth. Drug-free, infected or uninfected cells (wells B3 through G3) and reagent controls (B2 through G2) receive medium alone. The final 2 compounds are then transferred from wells H7 through H12 to a second test plate suing the same procedure. Test plates are incubated at 370C in Air-CO$_2$ for 7-14 days or until virus controls are lysed as determined macroscopically.

III. Quantitation of Viral Cytopathogenicity and Drug Activity

A. Materials

1. A solution of 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide. (XTT) - 1mg/ml solution in media without FCS. Store at 4°C. Prepare weekly.
2. Phenazine methosulfonate (PMS) stock solution - This can be prepared and maintained frozen until needed at -20°C. It should be made in PBS to a concentration of 15.3mg/ml.

B. Microculture Tetrazolium Assay (MTA)

1. Preparation of XTT-PMS Solution - The XTT-PMS is prepared immediately prior to its addition to the wells of the culture dish. The stock PMS solution is diluted 1:100 (0.153mg/ml). Diluted PMS is added to every ml of XTT required to give a final PMS concentration of 0.02mM. A 50µl aliquot of the XTT-PMS mixture is added to each of the appropriate wells, and the plate is incubated for four hours at 37°C. The plate lids are removed and replaced with adhesive plate sealers (Dynatech cat 001-010-3501). The sealed plate is shaken on a microculture plate mixer and the absorbance is determined at 450nm.

IV. Results

The data obtained were plotted as a percentage of test cells over uninfected cells (%) for both infected and uninfected cells as a function of the increasing concentration of the test compound. Such plots permit the calculation of an effective concentration (EC_{50}) with respect to infected cells, an inhibitory concentration (IC_{50}) with respect to normal cells and a therapeutic index (TI_{50}).

The inhibitory concentrations against HIV determined as described above for the compounds of Examples 7, 9, 10, 11 and 14(b) are shown in Table 1 in µg/ml.

TABLE 1

Compound	Example	Cell Line	ED_{50}	ID_{50}	TI_{50}
9a	7	MT-2	2.3	50	21.4
13a	9	MT-2	0.41	6.97	17.3
14a	10	MT-2	0.15	100	667
15a	11	MT-2	2.9	> 125	> 42.7
(-) 14a	14 (b)	CEM	0.66	189	284

The compounds of Examples 5 and 8 also showed antiviral activity in this screen.

An earlier assay carried out on the compound of Example 10 at the Southern Research Insitute yielded a TI_{50} of about 200 when MT-2 cells were cultured with H9/HILV-IIIB.

(B) Activity against Feline Leukemia Virus

Antiviral screening for activity against FeLV-FAIDS was performed in 96-well plates (Corning) using 81C indicator cells in Iscove's Modified Dulbecco's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). Twenty hours prior to the assay, the plates were seeded with the 81C cells at 5 x 10^3 cells/well. On the day of the assay, the cells were pretreated for 30 minutes at 37°C with DEAE-dextran (25µg/ml) in 0.1ml Hanks balanced salt solution. This was removed and then 0.1ml of growth medium containing 32 $TCID_{50}$ of FeLV-FAIDS, or 0.1ml of growth medium alone, was added to each well. The virus was allowed to adsorb for 1 hour, then 0.1 ml of test or positive control compound (2',3'-dideoxycytidine; ddC), or growth medium was added. Plates were incubated at 37 °C. Cells were fed fresh growth medium containing compound on Day 4 post-infection. Culture medium was completely changed and replaced with fresh medium containing compound on Day 7 post-infectin. On Day 10 post-infection the cells were fixed with formalin, stained with 0.1% Coomassie Brilliant Blue R-250 and observed microscopically for CPE and drug cytotoxicity.

The compound of Example 10 had an ED_{50} of 1.9 µg/ml.

(C) Activity against Murine AIDS

Falcon 6-well tissue culture plates were seeded with 1.75×10^5 cells per well in total volume of 2.5ml EMEM containing 5% heat-inactivated FBS. Twenty hours after the cells were seeded, the medium was decanted and 2.5ml DEAE-dextran (25 µg/ml in phosphate-buffered saline) was added to each well. The cultures were incubated at 37 °C for 1 hour, after which the DEAE-dextran solution was decanted and the cell layers rinsed once with 2.5ml PBS. Normal cell controls were refed with 2.5ml medium alone (no virus or drug). Drug control cultures received 2.5ml of medium containing drug but no virus. Virus-infected control cultures received 0.5ml of the appropriate dilution of stock CAS-BR-M to produce countable plaques plus 2.0ml medium. The test samples received 0.5ml of the appropriate virus dilution plus 2.0ml medium of the drug dilution. Six concentrations of the test compound diluted in serial half-$\log_{10}$ dilutions were tested. Three concentrations of the positive control drug, ddC, were tested. Triplicate wells for each concentration of test compound and 6 virus and 6 cell control cultures were included in each assay. On Day 3 post-virus inoculation toxicity of the drug for the SC-1 cells was determined by microscopic examination of stained duplicate cell and drug control cultures. The remaining test and control cultures were irradiated with an ultraviolet lamp for 20 seconds and XC cells were added to each culture (5×10^5 cells/well in 2.5ml EMEM containing 10% heat-inactivated FBS). On Day 3 post-UV irradiation, the cultures were fixed with formalin and stained with crystal violet. The plaques were counted with the aid of a dissection microscope.

Antiviral activity in the CAS-BR-M plaque reduction was expressed in terms of the reduction in the mean number of plaques counted in the drug-treated, virus-infected cultures compared with the mean number of plaques counted in the untreated, virus-infected control cultures (percent of control). The compound of Example 10 had an ED_{50} of 1.1μg/ml.

(D) Activity against Simian retrovirus SAIDS (SRV-2)

Antiviral screening against the SAIDS virus (D/Washington) was performed by a syncytia-inhibition assay on Raji cells. The drug was diluted in complete Iscove's medium and then 100μl of each dilution was added to the appropriate wells of a 96-well plate. Actively growing Raji cells, 5×10^3 cells in 50μl of complete Iscove's medium, were then added to each well. This was followed by the addition of 50μl of clarified supernate from an SRV-2/Raji cell co-culture. DDC was included in this assay as the positive control drug. Plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Syncytia were counted on Day 7 post-infection. Drug toxicity was ascertained by comparing viable cell counts of the uninfected, drug-treated sample to the viability of the uninfected, untreated control. The compound of Example 10 had an ED_{50} of 2.8μg/ml.

(E) Activity against Visna Maedi Virus

The antiviral activity against Visna Maedi Virus (VMV) strain WLC-1, was determined by measuring reduction of virus-specific immunohistochemical staining. Monolayers of sheep choroid plexus cells were infected with VMV and overlaid with serial dilutions of test compounds. After incubation for five days, the monolayers were further incubated with virus specific antisera conjugated to horse radish peroxidase (HRP). Subsequent incubation of the monolayers with a chromagenic substrate of HRP, strains areas of virus replication. These discrete foci were counted and the concentration of test compound required to reduce the number of foci to 50% of that of drug untreated controls calculated.

The compound of Example 13 had an ED_{50} 0.2μg/ml.

Example 22

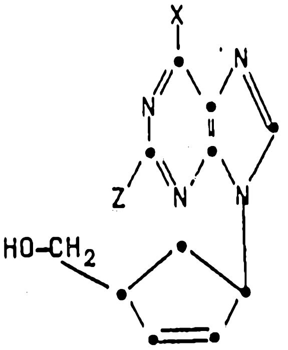
CYTOTOXIC ACTIVITY

The compounds of Examples 5, 7 and 8 showed cytotoxic activity when tested against P388 mouse leukemia cell culture assay as described by R. G. Alonquist and R. Vince, J. Med. Chem, 16, 1396 (1973). The ED_{50}'s (μg/ml) obtained were :-

Example 5	12
Example 7	40
Example 8	3

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A compound of formula (I)



(I)

wherein X is hydrogen, NRR^1, SR, OR or halogen;
Z is hydrogen, OR^2 or NRR^1;
R, R^1 and R^2 may be the same or different and are selected from hydrogen, C_{1-4} alkyl and aryl as herein before defined;
and pharmaceutically acceptable derivatives thereof as hereinbefore defined.

2. A compound of formula (I) according to claim 1 and pharmaceutically acceptable salts thereof.

3. A compound according to claim 1 or claim 2 wherein in the compound of formula (I) Z is H, OH or NH_2.

4. A compound according to any one of claims 1 to 3 wherein Z is NH_2.

5. A compound according to any one of claims 1 to 4 wherein X is hydrogen, chloro, NH_2, SH or OH.

6. A compound according to any one of claims 1 to 5 wherein X is OH.

7. A compound according to any one of claims 1 to 5 wherein Z is H or NH_2.

8. A compound selected from:-

(1α,4α) -4-(6-Chloro-9H-purin-9-yl)-2-cyclopentenyl carbinol;

(1α,4α) -4-(6-Hydroxy-9H-purin-9-yl)-2-cyclopentenyl carbinol;

(1α,4α) -4-(6-Amino-9H-purin-9-yl)-2-cyclopentenyl carbinol;

(1α,4α) -4-(6-Mercapto-9H-purin-9-yl)-2-cyclopentenyl carbinol;

(1α,4α) -4-(2,6-Diamino-9H-purin-9-yl)-2-cyclopentenyl carbinol;

(1α,4α) -4-(2-Amino-6-chloro-9H-purin-9-yl)-2-cyclopentenyl carbinol; and

(1α,4α) -4-(2-Amino-9H-purin-9-yl)-2-cyclopentenyl carbinol.

9. (1α,4α) -4-(2-Amino-6-hydroxy-9H-purin-9-yl)-2-cyclopentenyl carbinol.

10. A compound according to any one of claims 1 to 9 in the form of substantially a racemic mixture.

11. A compound according to any one of claims 1 to 9 consisting substantially of an optical isomer.

12. A compound according to any one of claims 1 to 9 consisting substantially of the D isomer.

13. A method of treating viral infection in an animal including a human comprising administering to said animal an effective amount of a compound of formula (I) as defined in any one of claims 1 to 12.

14. A pharmaceutical formulation comprising a compound of formula (I) as defined in any one of claims 1 to 12 or a pharmaceutically acceptable derivative thereof together with a pharmaceutically acceptable carrier therefor.

~~15. A pharmaceutical formulation comprising a compound of formula (I) as defined in any one of claims 1 to 12 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier therefor.~~

15. A pharmaceutical formulation according to claim 14 additionally comprising a further therapeutic agent.

Dated this 7th day of January 1992

REGENTS OF THE UNIVERSITY OF MINNESOTA
By Its Patent Attorneys
DAVIES COLLISON CAVE