

**COMMONWEALTH OF AUSTRALIA**  
**PATENTS ACT 1952**  
**APPLICATION FOR A STANDARD PATENT**

Eli Lilly and Company, a corporation organised and existing under the laws of the State of Indiana, of Lilly Corporate Center, Indianapolis, Indiana, 46285, UNITED STATES OF AMERICA, hereby apply for the grant of a standard patent for an invention entitled:

2',3'-Dideoxy-2',2'-difluoronucleosides

which is described in the accompanying complete specification.

Details of basic application(s):-

<u>Basic Applic. No:</u>	<u>Country:</u>	<u>Application Date:</u>
156116	US	16 February 1988

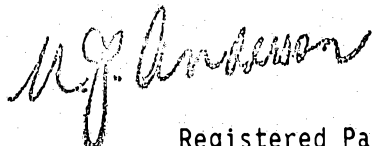
The address for service is:-

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DATED this FOURTEENTH day of FEBRUARY 1989

Eli Lilly and Company

By:



Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS  
OUR REF: 86230  
S&F CODE: 53710

APPLICATION ACCEPTED AND AMENDMENTS

ALLOWED ..... 13/12/90

5005452 14/02/89

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made for a patent for an invention entitled:

2',3'-Dideoxy-2',2'-difluoronucleosides

I/We, ..... Mary Ann Tucker, Lilly Corporate Center, City of .....  
 [full name of declarant(s)]

of ..... Indianapolis, Indiana 46285, United States of America .....  
 [full address of declarant(s) - not post office box]

.....  
 do solemnly and sincerely declare as follows:-

1. I am/~~we are~~ authorised by Eli Lilly and Company, the applicant for the patent to make this declaration on its behalf.
2. The basic application as defined by Section 141 of the Act was made in United States of America on 16 February 1988 by Larry W. Hertel.
3. LARRY WAYNE HERTEL of 912 Whidbey Court Indianapolis, Indiana 46229; CORA SUE GROSSMAN of 5838 Baron Court Indianapolis, Indiana 46250, and JULIAN STANLEY KROIN of 8418 Hilltop Drive Indianapolis, Indiana 46234, all in United States of America are the actual inventors of the invention and the facts upon which the applicant is entitled to make the application are as follows:

Larry Wayne Hertel is the inventor of the invention disclosed in the basic application and the present application in Australia includes additional matter not in the basic application the inventors of which were Cora Sue Grossman and Julian Stanley Kroin. The said Eli Lilly and Company is the assignee of all rights in and to the invention including the priority rights of the basic application from the said inventors.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Indianapolis, Indiana this 22nd day of February 1989  
 ELI LILLY AND COMPANY

.....  
 Signature of Declarant

TO: THE COMMISSIONER OF PATENTS  
 AUSTRALIA

Mary Ann Tucker  
 Assistant Patent Counsel

**(12) PATENT ABRIDGMENT (11) Document No. AU-B-29926/89**  
**(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 607840**

(54) Title  
**2',3'-DIDEOXY-2',2'-DIFLUORONUCLEOSIDES**

International Patent Classification(s)  
(51)<sup>4</sup> C07D 405/04 A61K 031/52 A61K 031/53 C07D 307/22  
C07D 473/04 C07D 473/18 C07D 473/30 C07D 473/34  
C07D 473/40 A61K 031/505  
(51)<sup>5</sup> C07H 019/09 C07H 019/12 C07H 019/19 C07H 019/073  
C07H 019/173

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(71) Applicant(s)  
**ELI LILLY AND COMPANY**

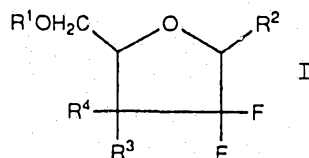
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(74) Attorney or Agent  
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(56) Prior Art Documents  
AU 581269 50555/85 C07H 019/073, 019/12, 019/16  
AU 590462 82659/87 C07D 405/04, 473/16, 473/18

(57) Claim

1. A compound of the formula

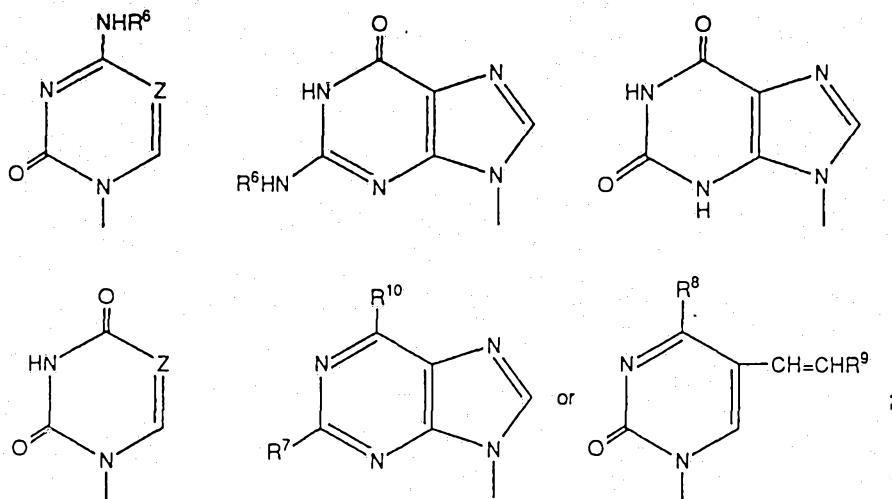


wherein:

R<sup>1</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl or  $\begin{matrix} \text{O} \\ || \\ -\text{C}-\text{R}^5 \end{matrix}$ ;  
R<sup>2</sup> is a base defined by one of the formulae

(11) AU-B-29926/89  
(10) 607840

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R<sup>3</sup> is hydrogen, amino, azido or fluoro;

R<sup>4</sup> is hydrogen or fluoro;

each R<sup>5</sup> independently is hydrogen or C<sub>1</sub>-C<sub>4</sub>

alkyl;

R<sup>6</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl or  $\begin{array}{c} \text{O} \\ || \\ -\text{C}-\text{R}^5 \end{array}$ ;

R<sup>7</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> haloalkyl, amino, bromo, fluoro, chloro or iodo;

R<sup>8</sup> is hydroxy or amino;

R<sup>9</sup> is hydrogen, bromo, chloro or iodo;

R<sup>10</sup> is -NHR<sup>6</sup>, bromo, chloro, hydroxy, fluoro or iodo;

Z is N or C-R<sup>7</sup>; or

a pharmaceutically-acceptable salt thereof;

with the proviso that when R<sup>4</sup> is fluoro, R<sup>3</sup>

is other than amino or azido.

13. A method of treating susceptible neoplasma in a mammal requiring such treatment, comprising administering to said mammal an antineoplastically effective amount of a compound of any one of claims 1 to 5, 8 or 9 and/or a pharmaceutical formulation of any one of claims 6, 11 or 12.

14. A method of treating viral infections in a mammal requiring such treatment, comprising administering to said mammal an antivirally effective amount of a compound of any one of claims 1 to 5, 8 or 9 and/or a pharmaceutical formulation of any one of claims 6, 11 or 12.

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FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE:

Class Int Class

Complete Specification Lodged:  
Accepted:  
Published:

Priority:

Related Art:

This document contains the  
amendments made under  
Section 49 and is correct for  
printing.

Name and Address  
of Applicant:

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Complete Specification for the invention entitled:

2',3'-Dideoxy-2',2'-difluoronucleosides

The following statement is a full description of this invention, including the best method of performing it known to me/us

## 2',3'-DIDEOXY-2',2'-DIFLUORONUCLEOSIDES

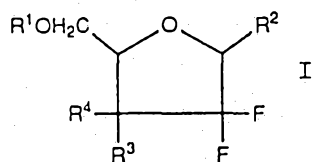
This invention relates to a novel class of compounds useful in the treatment of cancer.

5 While the treatment of cancer was once considered impossible, great strides have been made during the past ten years in controlling the ravages of this often fatal disease. Several drugs which contribute to the increasing survival rate of patients diagnosed as  
10 having one of several types of cancer are now routinely used clinically. The most commonly employed antitumor drugs include methotrexate, doxorubicin, cytarabine and the vinca alkaloids such as vincristine. However,  
15 research continues to develop more effective compounds with improved efficacy and greater safety for subjects under treatment for cancer.

The search for chemical compounds with oncolytic activity has revealed a class of 2'-deoxy-2',2'-difluoronucleosides which exhibit excellent  
20 activity against a variety of tumors, both solid and non-solid types, as disclosed in European Patent Application 85308547.0. These compounds, and their use as antiviral agents, are also disclosed in U.S. Patent No. 4,692,434.

25 The variety of injurious tumors which afflict humans, and the evolving nature of tumors, as well as the toxicity of many presently used oncolytic agents, call for continued effort to invent improved oncolytic agents. This invention discloses further new  
30 anticancer drugs.

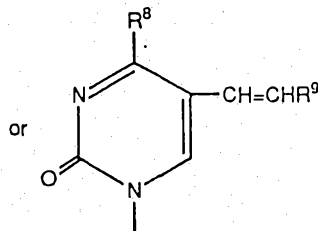
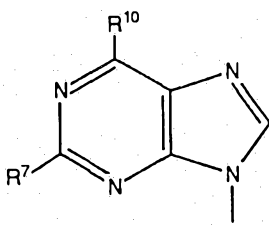
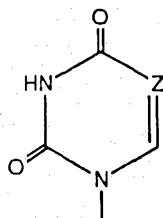
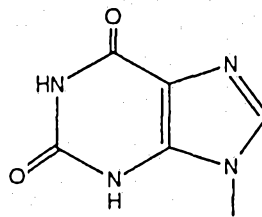
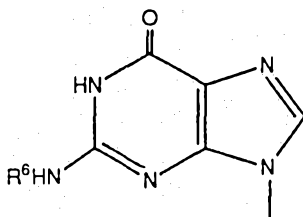
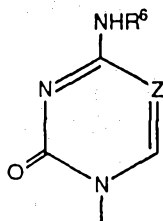
The present invention provides a new class of 2',3'-dideoxy-2',2'-difluoronucleosides useful for treating susceptible neoplasms in mammals and for treating viral infections in mammals. More specifically, the present invention relates to a compound of the formula



wherein:

$R^1$  is hydrogen,  $C_1-C_4$  alkyl or  $-C(=O)-R^5$ ;

$R^2$  is a base defined by one of the formulae



30

R<sup>3</sup> is hydrogen, amino, azido or fluoro;  
R<sup>4</sup> is hydrogen or fluoro;  
each R<sup>5</sup> independently is hydrogen or C<sub>1</sub>-C<sub>4</sub>

alkyl;

5

R<sup>6</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl or  $\begin{array}{c} \text{O} \\ || \\ -\text{C}-\text{R}^5 \end{array}$ ;

10

R<sup>7</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> haloalkyl,  
amino, bromo, fluoro, chloro or iodo;

R<sup>8</sup> is hydroxy or amino;

R<sup>9</sup> is hydrogen, bromo, chloro or iodo;

R<sup>10</sup> is -NHR<sup>6</sup>, bromo, chloro, hydroxy, fluoro  
or iodo;

15

Z is N or C-R<sup>7</sup>; or

a pharmaceutically-acceptable salt thereof;  
with the proviso that when R<sup>4</sup> is fluoro, R<sup>3</sup>  
is other than amino or azido.

20

The present invention also provides a pharmaceutically-acceptable salt thereof,  
associated with one or more pharmaceutically acceptable  
carriers, diluents or excipients therefor.

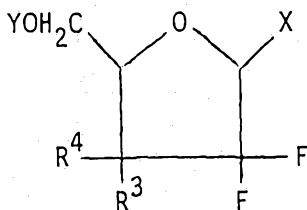
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Further embodiments of the invention are  
methods for treating susceptible neoplasms in mammals  
and for treating viral infections in mammals employing  
a compound of Formula I, or a pharmaceutically-acceptable  
salt thereof.



Intermediate compounds in the preparation of the compounds of the invention may be of the formula:

5



II

10 wherein:

X is hydroxy or a leaving group;

Y is hydrogen or a hydroxy protecting group;

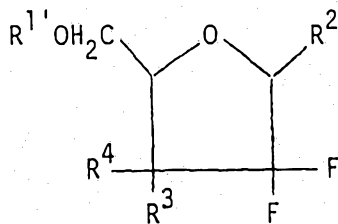
R<sup>3</sup> is hydrogen, amino, azido or fluoro; and

R<sup>4</sup> is hydrogen or fluoro;

15 with the proviso that when R<sup>4</sup> is fluoro, R<sup>3</sup> is other than amino or azido.

The invention further provides a process for preparing a compound of formula I wherein R<sup>1</sup> is hydrogen comprising (A) deblocking a compound of the formula

20



III

25 wherein:

R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined above; and

R<sup>1'</sup> is a hydroxy-protecting group;

30 or (B) reacting a compound of formula I wherein R<sup>1</sup> is hydrogen with an acidic salt-forming reagent.



In the above formula, the term C<sub>1</sub>-C<sub>4</sub> alkyl represents a straight or branched alkyl chain bearing from one to four carbon atoms. Typical C<sub>1</sub>-C<sub>4</sub> alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec.-butyl and t-butyl.

Halo represents fluoro, chloro, bromo or iodo.

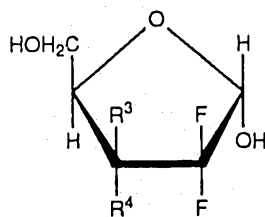
C<sub>1</sub>-C<sub>4</sub> Haloalkyl represents a C<sub>1</sub>-C<sub>4</sub> alkyl group having one or more halogen substituents. Typical C<sub>1</sub>-C<sub>4</sub> haloalkyl groups include trifluoromethyl, 2,2,3,3-tetrafluoroethyl, 4,4,4-trifluorobutyl, 2-chloroethyl, 3-bromopropyl and the like.

The term "hydroxy protecting group", as used herein, represents that substituent which can be placed efficiently on a hydroxy group, and which can be removed easily when the reaction is complete. Suitable groups may be those described in standard textbooks, such as Chapter 3 of Protective Groups in Organic Chemistry, McOmie, Ed., Plenum Press, New York (1973); and Chapter 2 of Protective Groups in Organic Synthesis, Greene, John Wiley & Sons, New York (1981). Suitable hydroxy protecting groups commonly employed

include formyl,  $\begin{array}{c} \text{O} \\ || \\ -\text{C}-(\text{C}_1-\text{C}_4 \text{ alkyl}) \end{array}$ , 2-chloroacetyl, benzoyl, benzyl, diphenylmethyl, triphenylmethyl, 4-nitrobenzyl, phenoxy-carbonyl, C<sub>1</sub>-C<sub>4</sub> alkyl such as t-butyl, methoxy-methyl, tetrahydropyranyl, allyl, tetrahydrothienyl, 2-methoxyethoxymethyl, methoxyacetyl, phenoxyacetyl, isobutyryl, ethoxycarbonyl, and benzyloxycarbonyl.

Silyl hydroxy-protecting groups are particularly convenient because most of them are cleaved easily by contact with water or an alcohol. Such groups may include especially trimethylsilyl, as well as isopropyl-  
5 dimethylsilyl, methyldiisopropylsilyl, t-butyldimethylsilyl or triisopropylsilyl.

The structural drawings defining the compounds of the present invention do not indicate their stereochemistry. Compounds of all configurations are believed to be useful, and the stereochemistry of the compound is not to be construed as a limitation. The preferred compounds possess the configuration of naturally occurring ribose, for example,



The configuration at the juncture between the carbohydrate and the base is preferably the  $\beta$  configuration as illustrated by the following  
25



5

The following compounds further illustrate compounds contemplated within the scope of the present invention:

10

1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose

1-(4-amino-5-chloro-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2,3-trifluororibose

15

1-(4-amino-5-bromo-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose

1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose

20

1-(4-amino-5-iodo-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose

1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-3-amino-2,2-difluororibose

1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2,3-dideoxy-2,2-difluororibose

25

1-(6-amino-9H-purin-9-yl)-2,3-dideoxy-2,2-difluororibose

1-(4-amino-5-fluoro-2-oxo-1H-pyrimidin-1-yl)-  
2,3-dideoxy-2,2-difluororibose

1-(4-amino-5-chloro-2-oxo-1H-pyrimidin-1-yl)-  
2,3-dideoxy-3-azido-2,2-difluororibose

5 1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-  
2,3-dideoxy-2,2,3,3-tetrafluoroxylose

1-(2-amino-6-oxo-1H,9H-purin-9-yl)-3-amino-  
2,3-dideoxy-2,2-difluororibose

10 1-(6-amino-9H-purin-9-yl)-3-azido-2,3-dideoxy-  
2,2-difluororibose

1-(6-bromo-2-chloro-9H-purin-9-yl)-2,3-  
dideoxy-2,2-difluororibose

1-(2-chloromethyl-6-iodo-9H-purin-9-yl)-2,3-  
dideoxy-2,2,3-trifluororibose

15 or a pharmaceutically-acceptable salt  
thereof.

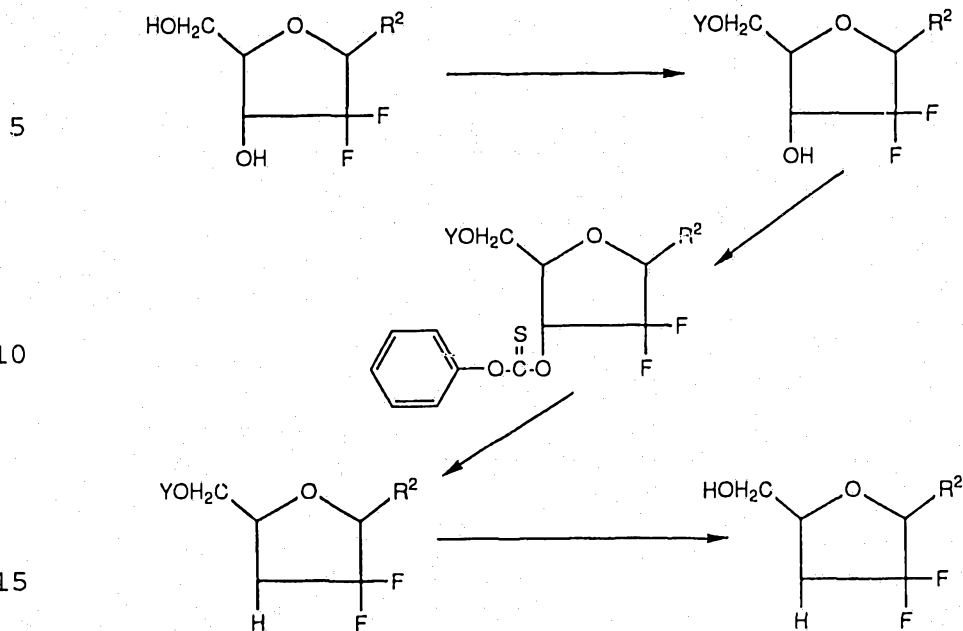
All of the above-described compounds of  
formula I are useful, of course, but certain compounds  
are preferred. Such preferred compounds are described  
20 by the following limitations. It will be understood  
that further preferred subclasses of compounds are to  
be obtained by combining the following limitations.

- 25 a.  $R^3$  and  $R^4$  are independently fluoro or  
hydrogen;
- b.  $R^3$  is amino or azido;
- c.  $R^6$  is hydrogen;
- d.  $R^5$  is alkyl;
- e.  $R^7$  is hydrogen or amino;
- f.  $R^{10}$  is  $-NHR^6$ ;
- 30 g.  $R^{10}$  is hydroxy.

As pointed out above, this invention includes the pharmaceutically acceptable salts of the compounds defined by the above formula. Since the compounds of this invention are basic in nature they react with any number of inorganic and organic acids to form pharmaceutically acceptable salts. Since the free amines of the invention are typically oils at room temperature or solids with low melting points, it is preferable to convert the free amines to their corresponding pharmaceutically acceptable salts, which are routinely solid at room temperature, for ease of handling and administration. Further, since salts of the compounds of the present invention are typically more water soluble than their corresponding free amines, these salts may be preferred in an effort to increase bioavailability of the active agent following administration. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic and acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caprate, heptanoate, propiolate, oxalate, malonate, succinate, suberate,

sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-  
1,6-dioate, benzoate, chlorobenzoate, methylbenzoate,  
dinitrobenzoate, hydroxybenzoate, methoxybenzoate,  
phthalate, sulfonate, xylenesulfonate, phenylacetate,  
5 phenylpropionate, phenylbutyrate, citrate, lactate,  
 $\beta$ -hydroxybutyrate, glycollate, maleate, tartrate,  
methanesulfonate, propanesulfonate, naphthalene-1-  
sulfonate, naphthalene-2-sulfonate, mandelate and the  
like salts. Preferred pharmaceutically acceptable  
10 salts include those formed with mineral acids such as  
hydrochloric acid and hydrobromic acid, and those formed  
with organic acids such as oxalic acid and maleic acid.

The nucleosides of the invention wherein  
both  $R^3$  and  $R^4$  are hydrogen are prepared by reacting a  
15 2'-deoxy-2',2'-difluoronucleoside with a reagent capable  
of protecting any free hydroxy and amine substituents  
other than the hydroxy group at the 3'-position on the  
carbohydrate moiety. The protected compound is next  
reacted with a phenyl halothionocarbonate to provide the  
20 corresponding 3'-[(phenoxythioxomethyl)oxy]-2',2'-  
difluoronucleoside, which is treated with tributyltin  
hydride and an azobismethylpropionitrile. The resulting  
compound is finally deblocked to provide the corre-  
sponding 2',3'-dideoxy-2',2'-difluoronucleoside of  
25 the invention. This reaction is represented by the  
following scheme:



20 wherein  $R^2$  is as defined above and Y is a hydroxy protecting group.

In the first step of the above-described reaction, the free hydroxy and amine substituents are protected without effect on the hydroxy group at the 3'-position of the carbohydrate moiety. According to this procedure, an equimolar or excess amount of a blocking agent such as pivaloyl chloride is added to a solution of the starting material in a suitable solvent

25



such as pyridine. This reaction is conducted under standard acylation techniques in the presence of a small amount of acylation catalyst such as dimethylamino-pyridine if desired. The product is isolated typically  
5 by concentrating the reaction mixture under vacuum and dissolving the resulting residue in a water immiscible organic solvent such as ethyl acetate or diethyl ether. The reaction mixture is washed with one or more aqueous solutions and concentrated under vacuum to provide the  
10 desired compound which may be further purified by standard techniques or taken directly for use in the following reaction.

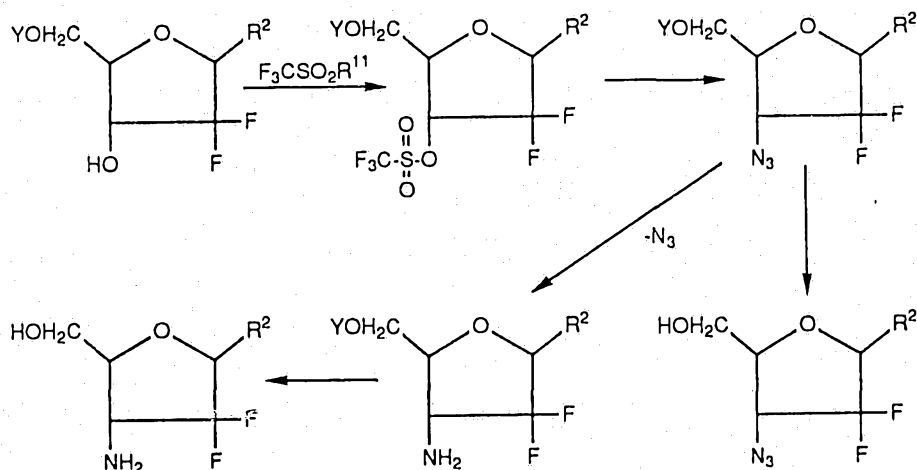
Alternatively, the starting compound may be reacted with a blocking agent such as benzoyl chloride,  
15 which reacts with the 3'-position hydroxy as well as the other groups to be protected. Then the 3'-benzoyl is selectively removed, as by hydrolysis with a strong base such as a sodium alkoxide, particularly potassium  
20 t-butoxide.

The hydroxy substituent at the 3'-position of the carbohydrate moiety is next converted to the 3'-phenoxythiocarbonyl ester according to procedures used previously on 2'-deoxyribonucleosides. See Journal of American Chemical Society 103, 932 (1981). According  
25 to this procedure, approximately an equimolar to slight excess of a phenyl halothionocarbonate is added to the starting material dissolved in a mutual solvent such as

pyridine. A small amount of acylation catalyst such as dimethylaminopyridine may also be employed. The compound thus synthesized is reacted with tributyltin hydride in the presence of 2,2'-azobis[2-methylpropionitrile] in a suitable organic solvent such as toluene. The reaction is substantially complete when conducted at a temperature in the range of about 50°C to about 150°C for a period of about 30 minutes to about 12 hours. The product is isolated by concentrating the reaction mixture under vacuum and isolating the desired product according to standard procedures. The desired compound is finally synthesized by hydrolyzing the protecting groups in the presence of a concentrated base such as sodium hydroxide or any other alkali metal hydroxide reagent. The product is isolated according to standard procedures and purified by common techniques such as crystallization from common solvents or purification over solid supports such as silica gel or alumina, or by reverse phase C<sub>18</sub> chromatography, to provide the desired compound.

Compounds of the invention wherein R<sup>3</sup> is amino or azido and R<sup>4</sup> is hydrogen are prepared according to known procedures. The hydroxy substituent at the 3'-position of a blocked 2'-deoxy-2',2'-difluoroxyllose as described above is blocked with a trifluorosulfonyl substituent. The resulting intermediate is reacted sequentially with a base, an azide reagent, catalytically hydrogenated and deblocked to provide the

corresponding compound of the invention wherein  $R^3$  is amino and  $R^4$  is hydrogen. The azide intermediate may be deblocked to provide a compound of the invention wherein  $R^3$  is azido and  $R^4$  is hydrogen. This reaction is represented by the following scheme:



wherein  $R^2$  is as defined above and  $R^{11}$  is a good leaving group such as halogen, especially chloro.

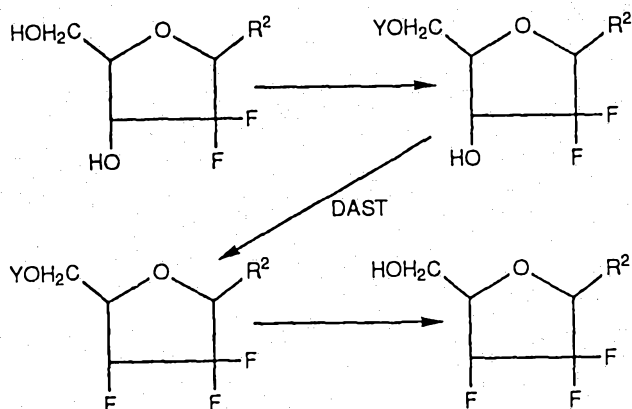
The reaction of a blocked 2'-deoxy-2',2'-difluoronucleoside with the trifluorosulfonyl reagent is carried out according to standard blocking conditions.

The preferred trifluorosulfonyl reagent will contain a

halogen substituent, with trifluorosulfonyl chloride being the especially preferred reagent. Typically an excess of such reagent is combined with a solution of the starting material dissolved in a mutual organic solvent. This reaction is complete within about 12 to 24 hours and it generally is conducted at a temperature from about 20°C to about 100°C. The desired product may be isolated, if desired, by standard techniques.

The 3'-trifluorosulfonyloxy derivative thus prepared is next reacted with an equimolar to excess amount of an azide reagent for conversion to the appropriate 3'-azido derivative. Typical azide reagents include trimethylsilyl azide as well as the alkali metal azides such as lithium azide, sodium azide or potassium azide. The reaction is carried out in a suitable solvent under anhydrous conditions at a temperature in the range of about 100°C to about -100°C. Suitable solvents include the aprotic solvents with N,N-dimethylformamide being preferred. The desired compound is preferably deblocked employing standard hydrolysis conditions in acid or base or converted to the 3'-amino derivative under standard catalytic hydrogenation conditions which is subsequently deblocked.

Compounds of the present invention wherein one of R<sup>3</sup> and R<sup>4</sup> is hydrogen and the other is fluoro are prepared by reacting a protected 2'-deoxy-2',2'-difluoronucleoside with diethylaminosulfur trifluoride (DAST) and deprotecting the resulting compound according to the following scheme:



wherein R<sup>2</sup> is as defined above and Y is a hydroxy protecting group.

15 In the first step of the above-described reaction, the free hydroxy and amine substituents of a 2'-deoxy-2',2'-difluoroxyllose are protected without effect on the hydroxy group at the 3'-position of the carbohydrate moiety. According to this procedure, an equimolar or excess amount of a blocking agent such as pivaloyl chloride is added to a solution of the starting material in a suitable solvent such as pyridine. This reaction is conducted under standard acylation techniques in the presence of a small amount of acylation catalyst such as dimethylaminopyridine if desired. The product is isolated typically by concentrating the reaction mixture under vacuum and dissolving the resulting residue in a water immiscible organic solvent such as ethyl acetate or diethyl ether. The reaction mixture is washed with one or more aqueous solutions and concentrated under vacuum to provide the desired compound

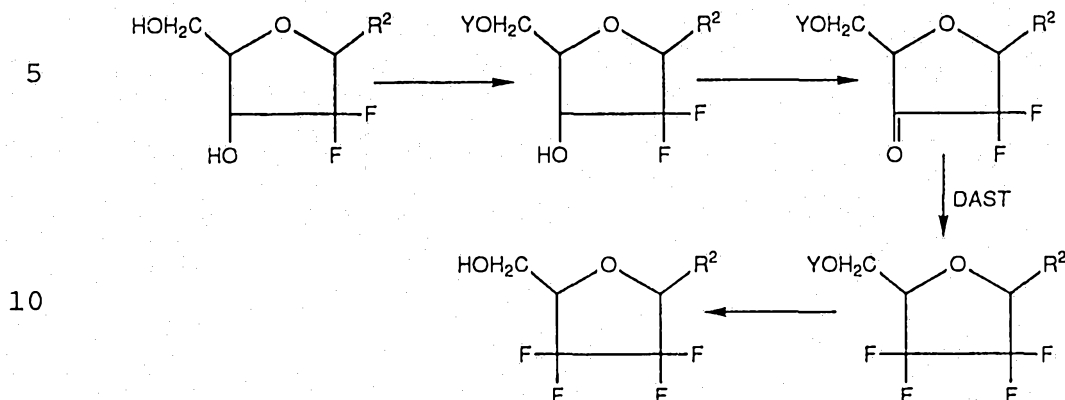
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which may be further purified by standard techniques or taken directly for use in the following reaction.

The hydroxy substituent at the 3'-position of the carbohydrate moiety is next converted to the fluoro substituent with DAST, a known fluorinating agent. See Journal of Organic Chemistry 40, 574 (1975) and Tetrahedron Letters, 573 (1977). According to this procedure, approximately an equimolar to slight excess of DAST is added to the starting material dissolved in a mutual solvent. The reaction is substantially complete when conducted at a temperature in the range of about 50°C to about 150°C for a period of about 30 minutes to about 12 hours. The product is isolated by concentrating the reaction mixture under vacuum and isolating the desired product according to standard procedures. The desired compound is finally synthesized by hydrolyzing the protecting groups in the presence of a concentrated base such as sodium hydroxide or any other alkali metal hydroxide reagent. The product is isolated according to standard procedures and purified by common techniques such as crystallization from common solvents or purification over solid supports such as silica gel or alumina, or by reverse phase C<sub>18</sub> chromatography, to provide the desired compound.

Compounds of the present invention wherein both R<sup>3</sup> and R<sup>4</sup> are fluoro are prepared by oxidizing the desired protected 2'-deoxy-2',2'-difluoronucleoside to provide the corresponding 3'-oxo-2',2'-difluoronucleoside which is treated with DAST and finally deprotected. This reaction may be represented by the following scheme:



wherein  $R^2$  is as defined above and Y is a hydroxy protecting group.

The protecting and deprotecting steps of the reaction, as well as the fluorination step with DAST, are all conducted according to the general conditions hereinbefore described. The process of oxidizing the 3'-hydroxy substituent to a 3'-oxo substituent is carried out according to standard conditions. Preferably, an equimolar to excess amount of dimethylsulfoxide-oxalyl chloride, also known as the Swern Reagent, is combined with the starting material in a suitable solvent such as methylene chloride according to Smith *et al.*, Synthesis 567 (1981). The reaction is complete within about one hour to about 48 hours when conducted

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at a temperature in the range of about  $-80^{\circ}\text{C}$  to about  $100^{\circ}\text{C}$ . The product is isolated and purified, if desired, according to standard procedures.

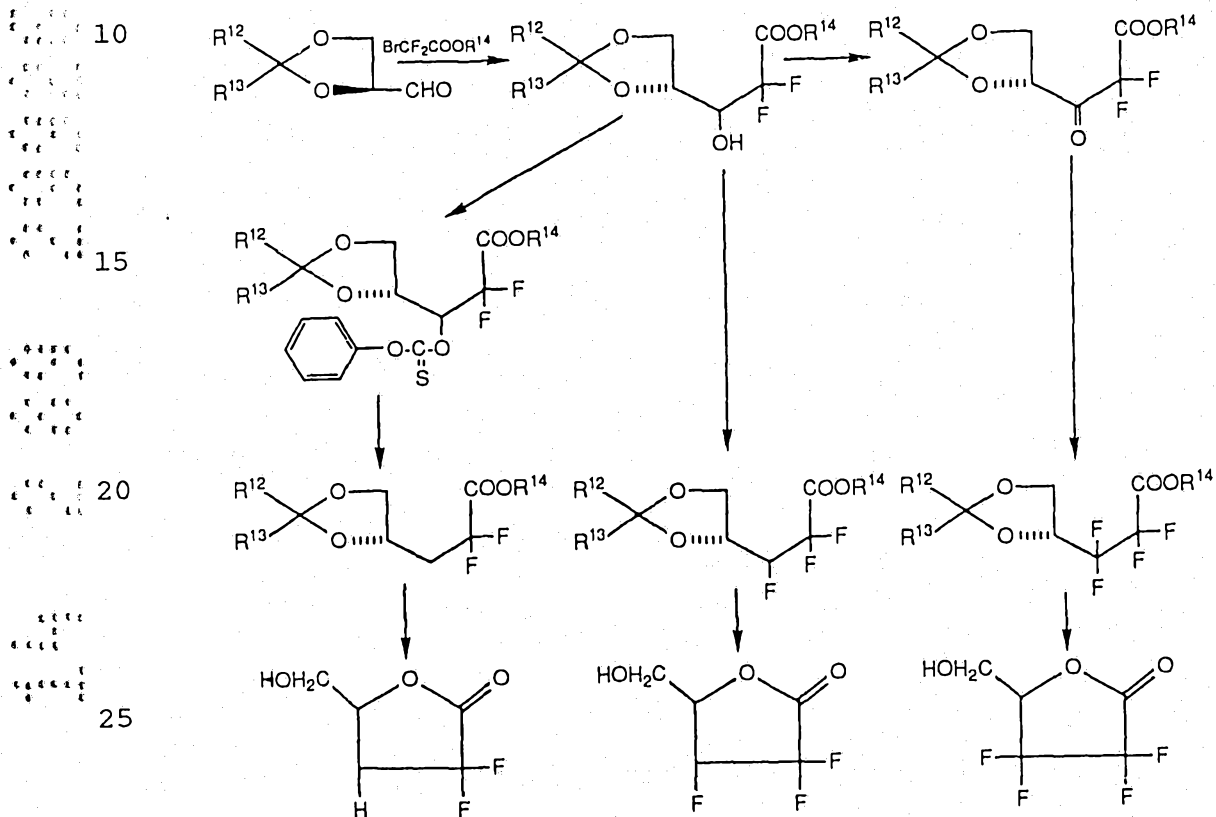
Intermediates ~~of the invention~~ as defined

5 by Formula II above may be prepared by procedures well known in the art or by procedures analogous to known processes and as hereinbefore provided. Intermediates wherein  $\text{R}^3$  and  $\text{R}^4$  are hydrogen or fluoro may be prepared by the reaction of D-glyceraldehyde ketonide and a  
10  $\text{C}_1\text{-C}_4$  alkylbromodifluoroacetate to provide an alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate according to the procedure of Hertel in U.S. Patent Nos. 4,526,988 and 4,692,434, herein incorporated by reference. Intermediates ~~of the invention~~ wherein  $\text{R}^3$  and  $\text{R}^4$  are both  
15 hydrogen are prepared by reacting the alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate with a phenyl halothionocarbonate to provide the corresponding intermediate which is treated with tributyltin hydride and an azobismethylpropionitrile. The resulting alkyl  
20 3-dioxolanyl-2,2-difluoropropionate is hydrolyzed under very mild conditions to provide the lactone form of the carbohydrate. Intermediates ~~of the invention~~ wherein one of  $\text{R}^3$  and  $\text{R}^4$  is hydrogen and the other is fluoro are prepared by fluorinating the alkyl 3-dioxolanyl-2,2-  
25 difluoro-3-hydroxypropionate to the alkyl 3-dioxolanyl-2,2,3-trifluoropropionate which is cyclized to the lactone form of the carbohydrate. For intermediates wherein  $\text{R}^3$  and  $\text{R}^4$  are both fluoro, the hydroxy substituent of the alkyl 3-dioxolanyl-2,2-difluoro-3-  
30 hydroxypropionate is oxidized to the keto derivative,



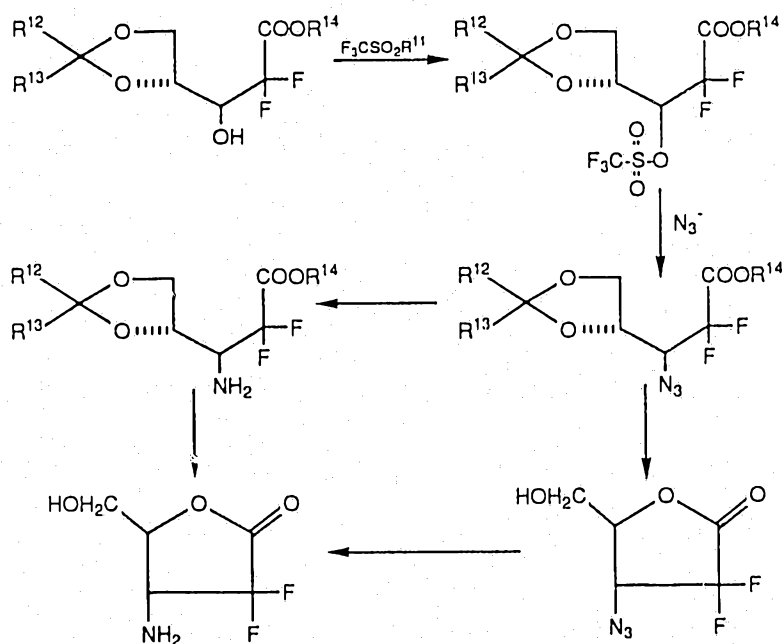


and the resulting compound is converted to an alkyl 3-dioxolanyl-2,2,3,3-tetrafluoropropionate. This compound is cyclized to provide the lactone form of the carbohydrate. The carbohydrate intermediates ~~of the~~  
 5 ~~invention~~ are finally prepared by reducing the keto oxygen of the lactone. This reaction is set forth by the following scheme:



wherein  $R^{12}$  and  $R^{13}$  are independently  $C_1$ - $C_3$  alkyl and  $R^{14}$  is  $C_1$ - $C_4$  alkyl.

Intermediates ~~of the invention~~ wherein  $R^3$  is amino or azido and  $R^4$  is hydrogen are prepared by reacting an alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate prepared as described above with a trifluorosulfonylhalide or other trifluorosulfonyl compound having a good leaving group to provide the corresponding trifluorosulfonyloxy substituted derivative which is converted to the azide. The azido derivative may be either cyclized to the lactone, which derivative may then be catalytically hydrogenated to the amine, or converted to the amine which is cyclized to the lactone. This reaction is represented by the following scheme:



wherein  $R^{11}$  is a good leaving group such as halogen,  $R^{12}$  and  $R^{13}$  are independently  $C_1-C_3$  alkyl, and  $R^{14}$  is  $C_1-C_4$  alkyl.

The conditions under which the foregoing  
5 propionate is prepared are the same as those herein-  
before provided. The lactone is prepared by hydrolyses  
of the propionate derivative. Proper control of the  
hydrolysis step will cleave the ketonide function and  
will also cleave the ester group, providing the lactone  
10 in a single step. The hydrolysis reagent is preferably  
a mildly acidic ion exchange resin, of which Dowex  
50W-X12 (Dow Chemical Company) is most highly preferred.  
It is possible to carry out the process with other mild  
hydrolytic reagents, although it is possible that larger  
15 amounts of by-products may be obtained. For example,  
aqueous acetic acid, or other relatively strong acids  
such as propionic acid, formic acid, chloroacetic acid,  
oxalic acid and the like, may be used for the hydrolysis.

The hydroxy groups of the lactone should be  
20 protected before its keto oxygen is reduced. The usual  
reaction conditions are used, depending on the nature  
of the protecting groups which may be chosen. For  
example, the t-butyl dimethylsilyl group is most con-  
veniently provided in the form of its trifluoromethane-  
25 sulfonate, and the protection reaction is carried out  
in the presence of a base such as lutidine, pyridine  
and the like. Acyl protecting groups such as acetyl,  
benzoyl and the like are provided by reacting the

lactone with an acylating agent such as an acyl chloride, bromide, cyanide or azide, or with an appropriate anhydride. The reactions are conveniently carried out in a basic solvent such as pyridine, quinoline or isoquinoline, or in a tertiary amine solvent such as triethylamine, tributylamine, methylpiperidine and the like. The reaction may also be carried out in an inert solvent, to which an acid scavenger, such as a tertiary amine, has been added. Acylation catalysts such as 4-dimethylaminopyridine or 4-pyrrolidinopyridine may be used in the reaction, if desired. The acylation reactions which provide protecting groups on the hydroxy groups are carried out at moderate temperatures in the range of from -25°C to 100°C. Such acylations may also be performed by acid-catalyzed reactions of the appropriate carboxylic acids, in inert organic solvents or neat. Acid catalysts such as sulfuric acid, polyphosphoric acid, methanesulfonic acid and the like are used.

Acyl protecting groups may also be provided by forming an active ester of the appropriate acid, such as the esters formed by such known reagents as dicyclohexylcarbodiimide, acylimidazoles, nitrophenols, pentachlorophenol, N-hydroxysuccinimide and 1-hydroxybenzotriazole.

Protecting groups of the ether type are placed by reacting the lactone with, for example, an appropriate diazo compound, such as diazomethane, phenyldiazomethane

or a silyldiazomethane. Such reactions are commonly and effectively carried out in solvents including esters such as ethyl acetate, halogenated solvents including dichloromethane and chloroform, and ethers including diethyl ether and tetrahydrofuran. The process is usually carried out at low temperatures from about  $-50^{\circ}\text{C}$  to about  $0^{\circ}\text{C}$ . Such ether-forming reactions may also be carried out with the assistance of reagents such as trimethyloxosulfonium hydroxide, trimethylsulfonium hydroxide and trimethylselenonium hydroxide, in solvents such as dimethylsulfoxide, dimethylformamide, hexamethylphosphoramide, acetone, acetonitrile and the like.

The silyl protecting groups discussed above are placed on the hydroxy groups by the conventional methods, such as by reaction with the appropriate silylcarboxamide or bis(substituted-silyl)carboxamide, or an appropriately substituted silazane. Suitably substituted silyl methanesulfonates, toluenesulfonates and the like are also useful. An equivalent of a base is usually necessary in the reaction mixture, unless a basic solvent such as is discussed above is used as the reaction medium.

When the hydroxy groups have been protected, the keto oxygen of the lactone is reduced to the alcohol, forming the protected 2,3-deoxy-2,2-difluororibose or xylose of this invention. The most preferred reducing agent is diisobutyl aluminum hydride, used at a low temperature in the range of about  $-100^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$ . It

is necessary to carry out the reduction very carefully, in order to avoid reducing conditions so vigorous that the ring is opened at the oxygen atom. Other metal hydrides, such as the widely used lithium aluminum  
5 hydride, can also be used for the reduction, but it is necessary to keep the temperature quite low and to assure that the hydride is destroyed before the temperature is allowed to rise toward ambient. Accordingly, a solvent with a very low freezing point must be used  
10 in the reduction step. Toluene is convenient; other solvents can of course be used, including lower alkanols, especially ethanol, ethers such as diethyl ether, and the like.

The nucleoside compounds of the present  
15 invention as defined by Formula I may also be prepared by first preparing the appropriately substituted carbohydrate moiety and then attaching the carbohydrate to the desired base according to standard procedures. An appropriate leaving group must be placed at the 1-  
20 position of the carbohydrate, in order to obtain efficient reaction with the base. The preferred leaving group is methanesulfonyl, which is readily provided by reaction with methanesulfonyl chloride in the presence of an equivalent amount of a suitable acid scavenger  
25 such as triethylamine and the like. Other sulfonyl leaving groups, particularly toluenesulfonyl, are provided in the same way by reaction with the appropriate sulfonyl halide.

When a chloro or bromo leaving group is to be used, it is frequently convenient to first make the 1-acetate derivative, for instance by reaction with acetic anhydride, or another source of acetyl groups, in the presence of an equivalent or more of an acid scavenger. Then the acetate group is displaced with gaseous hydrogen bromide or hydrogen chloride, at a low temperature such as about  $-50^{\circ}\text{C}$  to about  $0^{\circ}\text{C}$ . Since the gaseous hydrogen halide may tend to remove the protecting groups, especially silyl protecting groups, it is necessary to operate this step at quite a low temperature and to add the hydrogen halide slowly in small increments.

The bases used to form the compounds of the present invention are commonly known to organic chemists, and no discussion of their synthesis is necessary. However, the primary amino groups which are present on some of the bases should be protected before the base is coupled with the carbohydrate. The usual amino-protecting groups are used, including silyl groups such as have been discussed, as well as such typical groups as t-butoxycarbonyl, benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, formyl, acetyl, and the like.

It is often advisable to convert keto oxygen atoms on the bases to the enol form, in order to make the bases more highly aromatic and thereby allow more ready attack of the base by the carbohydrate. It is

most convenient to enolize the oxygens by providing silyl protecting groups for them. The usual silyl protecting groups as discussed above are used for this purpose, also.

5           The reaction between the protected carbohydrate and the base is preferably carried out neat at an elevated temperature in the range of from about 50°C to about 200°C. It is possible, however, to use  
10           relatively high-boiling solvents for the reaction, such as dimethylformamide, dimethylacetamide, hexamethylphosphoramide and the like. However, if the coupling reaction is carried out under elevated pressure, to avoid distillation of a low-boiling solvent, any convenient inert reaction solvent can be used.

15           The coupling reaction may be done at low temperatures if a reaction initiator, such as a trifluoromethanesulfonyloxysilane, is used. The usual inert reaction solvents, as discussed above, may be used at temperatures in the range of from about ambient  
20           to about 100°C.

          The final step of the reaction sequence is the removal of the protecting groups. Most silyl protecting groups are easily cleaved by contact with water or an alcohol. The t-butyldimethylsilyl protecting group requires acid conditions, such as contact  
25           with gaseous hydrogen halide, for its removal.

          Acyl protecting groups are removed by simple hydrolysis with strong or moderately strong bases, such



as alkali metal hydroxides, at temperatures from about the ambient temperature to about 100°. At least one equivalent of base is needed for each protecting group, of course. Such hydrolyses are conveniently carried  
5 out in hydroxylic solvents, especially aqueous alkanols. The reactions may be also carried out, however, in any convenient solvent, such as polyols including ethylene glycol, ethers such as tetrahydrofuran and the like, ketones such as acetone and methyl ethyl ketone and  
10 other polar solvents such as dimethylsulfoxide. The cleavage of acyl protecting groups may also be performed with other bases, including, for example, sodium methoxide, potassium t-butoxide, hydrazine, hydroxylamine, ammonia, alkali metal amides and  
15 secondary amines such as diethylamine and the like. The acyl protecting groups can also be removed with acid catalysts, such as methanesulfonic acid, hydrochloric acid, hydrobromic acid, sulfuric acid, or with acidic ion exchange resins. It is preferred to carry out such  
20 hydrolyses at a relatively high temperature, such as the reflux temperature of the mixture, but temperatures as low as ambient may be used when particularly strong acids are used.

The removal of protecting groups which are  
25 ethers is carried out by known methods, for example, with ethanethiol and aluminum chloride.

None of the reaction steps require unusual excesses of the reactants. As usual in organic

syntheses, it is advisable and economical to use a moderate excess, in the range of 1.05X to 2X, for example, of the cheaper reagents to assure that the costlier ones are consumed.

5           As has been noted above, the  $\beta$ -nucleosides of this invention are preferred. A particularly convenient enzymatic process has been discovered for isolating the  $\beta$ -nucleosides wherein  $R^2$  is 2-amino-6-oxo-9H-purine. The process is carried out by reacting the racemic  
10 mixture of  $\alpha$ - and  $\beta$ -nucleosides wherein  $R^2$  is 2,6-diamino-9H-purine with adenosine deaminase, preferably Type I adenosine deaminase. The enzyme preferentially deaminates the 6-position of the  $\beta$ -nucleoside.

15           In the above isolation process, a catalytic to approximately an equimolar or excess amount of adenosine deaminase is added to a solution of the appropriate starting material in a suitable solvent. While a variety of solvents may be used, preferred solvents include the polar solvents such as the  
20 alcohols or water, which is preferred. The reaction is substantially complete after about 10 minutes to about 12 hours when conducted at a temperature in the range of about 0°C to about 100°C. The reaction is preferably conducted for approximately 1 to 4 hours at  
25 a temperature in the range of about 20°C to about 25°C.

30           If the foregoing reaction is allowed to proceed beyond the approximate maximum time indicated, the quantity of  $\alpha$ -isomer produced will increase accordingly. Therefore, to maximize the amount of  $\beta$ -isomer synthesized it is preferred to follow the progress of

the reaction by high performance liquid chromatography or thin layer chromatography.

The desired  $\beta$ -difluoronucleoside prepared by either process is readily recovered by standard techniques, such as extracting the desired compound into an organic solvent or, preferably, collecting the precipitated solid by vacuum filtration. The desired compound may be further purified if desired by crystallization from common solvents or column chromatography over solid supports such as silica gel or alumina, and especially  $C^{18}$  high performance liquid chromatography. However, such additional purification is not usually needed.

The pharmaceutically acceptable acid addition salts of the invention are typically formed by reacting a 2',3'-dideoxy-2',2'-difluoronucleoside of the invention with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene, and the salt normally precipitates out of solution within about one hour to 10 days, and can be isolated by filtration.

The compounds employed as starting materials in the synthesis of the compounds of the invention are well known and readily synthesized by standard procedures commonly employed by those of ordinary skill in the art. The 1-substituted-2-deoxy-2,2-difluoro-ribose and -xylose derivatives are taught in U.S. Patent Nos. 4,526,988 and 4,692,434, herein incorporated by reference.

The following Examples further illustrate specific compounds of the present invention. The Examples are not intended to be limiting to the scope of the invention in any respect and should not be so construed.

Example 1

$\beta$ -1-(4-Amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose

A.  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-2-deoxy-2,2-difluororibose

A mixture of 0.9 g (0.003 mol) of  $\beta$ -1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-deoxy-2,2-difluororibose and 0.726 g (0.742 ml, 0.006 mol) of pivaloyl chloride in 10 ml of dry pyridine was refluxed for approximately 3½ hours with stirring. The pyridine was removed under vacuum at 45°C. The resulting suspension was dissolved in toluene and concentrated again under vacuum. The residue was dissolved in ethyl acetate and the resulting solution was washed once with water, once with 2N hydrochloric acid, once with a saturated sodium bicarbonate solution, once with water and finally once with a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum to provide a white residue. The residue was dissolved in 150 ml of boiling ethyl acetate which

was then concentrated to crystallization. The crystals were filtered and dried under vacuum at 50°C to provide 0.656 g of  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-2-deoxy-2,2-difluororibose.

B.  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-3-(O-phenylcarbonothiooxy)-2-deoxy-2,2-difluororibose

To a solution of 0.65 g (1.51 mmol) of  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-2-deoxy-2,2-difluororibose and 0.02 g of 4-dimethylaminopyridine (DMAP) in 13 ml of dry pyridine under a nitrogen atmosphere was added 0.286 g (0.23 ml, 1.66 mmol) of phenyl chlorothionocarbonate. The reaction mixture was stirred at room temperature overnight. A thin layer chromatograph in methylene chloride:methanol (19:1, v:v) indicated that no starting material was present. The reaction mixture was concentrated under vacuum at 50°C. The residue was combined with toluene and the resulting mixture was again concentrated under vacuum. The residue was dissolved in ethyl acetate and water and washed once with 2N hydrochloric acid, once with a saturated sodium bicarbonate solution, once with water and once with saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum to provide 0.87 g of  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-3-(O-phenylcarbonothiooxy)-2-deoxy-2,2-difluororibose

as a yellow orange solid.

C.  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-2,3-dideoxy-2,2-difluororibose

5 To a solution of 0.87 g (1.5 mmol) of  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-3-(O-phenylcarbonothiooxy)-2-deoxy-2,2-difluororibose and 0.03 g of  
10 2,2'-azobis[2-methylpropionitrile] (AIBN) in 19 ml of dry toluene under a nitrogen atmosphere was added 0.81 ml (3.1 mmol) of 3.79M tributyltin hydride. The reaction mixture was heated at 85°C for 3½ hours and an additional 0.12 ml of tributyltin hydride was added to the reaction mixture. The reaction mixture was stirred  
15 at 85°C for one hour and concentrated under vacuum at about 50°C. The residue was triturated twice with 15 ml of hexane. The hexane was decanted and the residue was concentrated under vacuum to provide a residue which was chromatographed over silicon dioxide employing  
20 methylene chloride:methanol (49:1, v:v). Fractions containing the major component were combined and the solvent was evaporated therefrom to provide 0.38 g of  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-2,3-dideoxy-  
25 2,2-difluororibose.

D. To a solution of 0.37 g (0.89 mmol) of  $\beta$ -1-[4-(2,2-dimethyl-1-oxopropylamino)-2-oxo-1H-pyrimidin-1-yl]-5-(2,2-dimethyl-1-oxopropoxy)-2,3-dideoxy-2,2-difluororibose in 20 ml of methanol was  
30 added 4 ml of concentrated ammonium hydroxide. The

reaction mixture was sealed in a glass vial. The mixture was heated at 50°C for 2 hours and then stirred at room temperature overnight. An additional 3 ml of ammonium hydroxide was added to the mixture. The mixture was heated at 60°C for 3 hours. The mixture was heated for 10 minutes at 100°C, cooled and concentrated under vacuum. The residue was dissolved in ethyl acetate and water. The layers were separated. The aqueous layer was concentrated under vacuum to provide an oil which was dissolved in acetone. The resulting solution was concentrated under vacuum to provide 0.16 g of the desired compound  $\beta$ -1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose as a white foam. The foam was chromatographed on a Whatman Partisil 10 ODS-3 column, 250 x 10 mm, with 9:1 water:methanol to yield 0.11 g of purified product.

$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  2.50 (m, 2H, 3'-H); 3.72 (d, 1H, 5B'-H); 3.90 (d, 1H, 5A'-H); 4.32 (m, 1H, 4'-H); 5.93 (d, 1H, 5-H); 6.25 (d, 1H, 1'-H); 8.02 (d, 1H, 6-H).

High resolution MS: obs 248.0847, calculated for  $\text{M}+1$ :  $\text{C}_9\text{H}_{12}\text{N}_3\text{O}_3\text{F}_2$ , 248.0847.

#### Example 2

$\beta$ -1-(2,6-diamino-9H-purin-9-yl)-2,3-dideoxy-2,2-difluororibose

To a solution of 0.2 g (0.5 mmol) of  $\beta$ -1-(2,6-diamino-9H-purin-9-yl)-5-benzoyl-2-deoxy-2,2-difluororibose and 6 mg (0.05 mmol) of 4-dimethylaminopyridine

in 5 ml of dry pyridine under a nitrogen atmosphere was added 0.38 g (0.3 ml, 2.2 mmol) of phenyl chlorothionocarbonate. The reaction mixture was stirred at room temperature overnight. A thin layer chromatograph in  
5 methylene chloride:methanol (19:1, v/v) indicated that no starting material was present. The reaction mixture was evaporated to dryness and the residue was combined with toluene and the resulting mixture was again concentrated under vacuum. The residue was dissolved in ethyl  
10 acetate and washed once with water, once with 1.0N hydrochloric acid, once with a 10% by volume sodium bicarbonate solution, once with water and once with saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate and concentrated  
15 under vacuum to provide 0.25 g of  $\beta$ -1-(2,6-diamino-9H-purin-9-yl)-5-benzoyl-3-(O-phenylcarbonothiooxy)-2-deoxy-2,2-difluororibose which was used without further workup. Mass spec. m/e = 542 = P.

To a solution of 0.25 g (.0.046 mmol) of the  
20 above intermediate and 10 mg of 2,2'-azobis[2-methylpropionitrile] in 10 ml of dry toluene under a nitrogen atmosphere was added 0.24 ml (0.92 mmol) of tributyltin hydride. The reaction mixture was heated at 85°C for 12 hours and an additional 0.06 ml (0.23 mmol) of  
25 tributyltin hydride was added to the reaction mixture. The reaction mixture was stirred at 85°C for 8 hours and concentrated under vacuum at about 50°C. The residue was taken up in acetonitrile and washed with hexane. The acetonitrile layer was concentrated under  
30 vacuum to provide an oil. The oil was chromatographed



over silicon dioxide employing methylene chloride:-  
methanol (19:1, v:v). Fractions containing the major  
component were combined and the solvent was evaporated  
therefrom to provide 20 mg of  $\beta$ -1-(2,6-diamino-9H-purin-  
9-yl)-5-benzoyl-2,3-dideoxy-2,2-difluororibose.  
5 Mass spec. m/e = 390 = P.

10 A solution of 20 mg (0.05 mmol) of the above  
intermediate in methanol (10 ml) was saturated with  
ammonia at 0°C. The solution was then warmed to  
ambient temperature and stirred overnight. Solvent was  
evaporated and the residue was chromatographed on silica  
gel, eluting with a gradient from 5% methanol in methyl-  
ene chloride to 10% methanol in methylene chloride.  
The product fractions were combined and evaporated to  
15 yield 6.3 mg of  $\beta$ -1-(2,6-diamino-9H-purin-9-yl)-2,3-di-  
deoxy-2,2-difluororibose. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz)  $\delta$   
2.55 (m, 1H, 3'A); 2.85 (m, 1H, 3'B); 3.7 (m, 1H, 5'A);  
3.9 (m, 1H, 5'B); 4.38 (m, 1H, 4'); 6.1 (m, 1H, 1');  
8.05 (s, 1H, H-8). Mass spec. m/e = 286 = P.

### 20 Example 3

$\beta$ -1-(2-amino-6-oxo-9H-purin-9-yl)-2,3-di-  
deoxy-2,2-difluororibose  
25

To a solution of 12 mg (0.042 mmol) of the  
compound of Example 2 in water (2 ml) at ambient  
temperature was added adenosine deaminase (Sigma). The  
reaction was followed by liquid chromatography (C18, 15%  
30 methanol in water, 1 ml/min) and enzyme was added

periodically until the reaction appeared to be complete. The reaction mixture was heated at reflux to deactivate the enzyme, and was then evaporated to a solid. The solid residue was recrystallized from D<sub>2</sub>O to yield 6.3  
5 mg of the desired product. <sup>1</sup>H NMR (D<sub>2</sub>O, 300 MHz) δ 2.67 (m, 2H, 3'); 3.75 (m, 1H, 5'A); 3.9 (m, 1H, 5'B); 4.5 (m, 1H, 4'); 6.1 (m, 1H, 1'); 8.0 (s, 1H, H-8). Mass spec. m/e = 287 = P.

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Example 4

β-1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-  
3-azido-2,3-dideoxy-2,2-difluororibose

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A suspension of 4.0 g (14 mmol) of β-1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-deoxy-2,2-difluororibose and 4.8 g (17 mmol) of triphenylmethyl chloride in 40 ml of dry pyridine were heated at reflux for 3 hours and then stirred overnight at ambient temperature. The reaction mixture was then poured into ice water and extracted three times with ether. The organic layer was washed with 1.0N hydrochloric acid and then with water and saturated aqueous sodium chloride, dried over sodium sulfate and evaporated under reduced pressure to yield 7.34 g of β-1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-triphenylmethyl-2-deoxy-2,2-difluororibose. Mass spec. m/e = 520 = P.

To a solution of 7.12 g (14 mmol) of the above intermediate in methylene chloride (248 ml) and pyridine (4.5 ml) was added 5.2 g (19 mmol) of triflic anhydride at 0°C. The reaction mixture was stirred at 5 0°C for 3 hours then evaporated under reduced pressure. The resulting residue was mixed with ethyl acetate and washed with cold water, saturated sodium bicarbonate, and saturated sodium chloride solution and evaporated to dryness. The residue was mixed with 10 toluene and stripped to dryness to yield 9.13 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-triphenylmethyl-3-trifluoromethylsulfonyl-2-deoxy-2,2-difluororibose.  $^1\text{H}$  Nmr ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  2.05 (s, 3H, 5- $\text{CH}_3$ ); 3.45 (m, 1H, 5'A); 3.7 (m, 1H, 5'B); 4.28 (m, 15 1H, 4'); 5.5 (m, 1H, 3'); 6.3 (m, 1H, 1'); 7.35 (m, 16H,  $\text{Ph}_3$  & H-6). Mass spec. m/e = 652 = P.

A solution of 9.13 g (14 mmol) of the above intermediate and 9.8 ml of 1.0N sodium hydroxide in ethanol (100 ml) was stirred at ambient temperature for 20 12 hours. The reaction mixture was neutralized with 1.0N hydrochloric acid and then the ethanol was evaporated resulting in a precipitate which was collected and dried to obtain 2.97 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-triphenylmethyl-3-(2-anhydro)-2-deoxy-2,2-difluororibose.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 25 300 MHz)  $\delta$  1.95 (s, 3H, 5- $\text{CH}_3$ ); 3.4 (m, 2H, 5'A&B); 4.5 (m, 1H, 4'); 4.85 (m, 1H, 3'); 5.3 (m, 1H, 1'); 6.9 (s, 1H, H-6); 7.3 (m, 15H,  $\text{Ph}_3$ ). Mass spec. m/e = 502 = P.

A suspension of 2.7 g (5.4 mmol) of the above intermediate in 80% acetic acid (54 ml) was heated at reflux for 2.5 hours. The solution was allowed to cool and the precipitate that formed was collected and identified as triphenylmethanol. The filtrate was evaporated to dryness and then mixed with water and extracted with hexane. The aqueous layer was evaporated to dryness to yield 1.65 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-deoxy-2,2-difluoroxylose.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz)  $\delta$  1.95 (s, 3H, 5- $\text{CH}_3$ ); 4.3 (series of M, 4H, 3', 4' & 5' A&B); 6.15 (dd, 1H, 1'); 7.6 (s, 1H, H-6). Mass spec. m/e = 278 = P.

A solution of 0.25 g (0.9 mmol) of the above intermediate, glacial acetic acid (5 ml) and water (0.06 ml, 3.3 mmol) was heated at reflux for 8 hours. The reaction mixture was allowed to cool and then evaporated to dryness under reduced pressure. The resulting residue was chromatographed on a silica gel column eluting with 5% methanol in methylene chloride. The product fractions were collected, combined and evaporated to dryness to yield 0.2 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-acetyl-2-deoxy-2,2-difluoroxylose.

To a solution of 0.1 g (0.3 mmol) of the above intermediate in pyridine (0.066 ml, 0.82 mmol) and methylene chloride (8 ml) at  $0^\circ\text{C}$  was added 0.115 g (0.41 mmol) triflic anhydride. The reaction mixture was stirred at  $0^\circ\text{C}$  for 1.5 hours at which time it was added to a mixture of ice and saturated sodium bicarbonate solution. The organic layer was separated,

washed with saturated sodium chloride, dried over sodium sulfate and evaporated to dryness to yield 0.14 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-acetyl-3-trifluoromethylsulfonyl-2-deoxy-2,2-difluoroxylose.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.95 (s, 3H, 5- $\text{CH}_3$ ); 2.05 (s, 3H,  $\text{COCH}_3$ ); 4.45 (m, 3H, 4' & 5' A & B); 5.3 (m, 1H, 3'); 6.35 (m, 1H, 1'); 7.05 (s, 1H, 6-H). Mass spec.  $m/e = 452 = P$ .

A solution of 0.13 g (0.28 mmol) of the above intermediate and 0.14 g (2.8 mmol) of lithium azide in dimethylformamide (5 ml) was stirred for 12 hours at  $-30^\circ\text{C}$ . The reaction was evaporated and the resulting residue was stripped twice with toluene. The residue was dissolved in ethyl acetate and washed with water, dried and evaporated under reduced pressure to yield 0.06 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-acetyl-3-azido-2,3-dideoxy-2,2-difluororibose. Mass spec.  $m/e = 345 = P$ . IR  $2120\text{ cm}^{-1}$ .

A solution of 0.06 g (0.17 mmol) of the above intermediate in a saturated solution of anhydrous ammonia in methanol (20 ml) was stirred at  $-8^\circ\text{C}$  for 2 hours. The solution was evaporated to dryness under vacuum at  $40^\circ\text{C}$ . The residue was chromatographed on silica gel eluting with 7% methanol in methylene chloride containing 1% v/v concentrated ammonium hydroxide. The main fraction was collected, combined and evaporated to dryness. The material was further purified by preparative HPLC using a C18 reverse phase column eluting with water/methanol, 1/1. The second compound to elute from the column was identified as

the desired product, (12.1 mg).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz)  $\delta$  1.75 (s, 3H, 5- $\text{CH}_3$ ); 3.7 (m, 1H, 5'A); 3.9 (m, 2H, 3'&5'B); 4.5 (m, 1H, 4'); 6.2 (apparent t, 1H, 1'); 7.7 (s, 1H, H-6). High resolution mass spec. obs.

5 304.08575, calculated for  $\text{M} + 1: \text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_4\text{F}_2$ ,  
304.08575. I.R. 2120  $\text{cm}^{-1}$ .

#### Example 5

10  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-  
2,3-dideoxy-2,2-difluororibose

To a solution of 0.2 g (0.63 mmol) of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3-pyrimidin-1-yl)-5-acetyl-2-deoxy-  
15 2,2-difluororibose and 0.02 g of 4-dimethylamino-  
pyridine in 5 ml of dry pyridine under a nitrogen atmosphere was added 0.13 g (0.10 ml, 0.75 mmol) of phenyl chlorothionocarbonate. The reaction mixture was stirred at room temperature overnight. A thin layer  
20 chromatograph in methylene chloride:methanol (19:1, v/v) indicated that no starting material was present. The reaction mixture was stripped three times with toluene. The residue was dissolved in ethyl acetate and water and washed once with 1.0N hydrochloric acid, once  
25 with 10% by volume sodium bicarbonate solution, once with water and once with saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum to provide

0.47 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-acetyl-3-(O-phenylcarbonothiooxy)-2-deoxy-2,2-difluororibose which was used without further workup.

To a solution of 0.47 g (0.63 mmol) of the  
5 above intermediate and 0.02 g of 2,2'-azobis[2-methylpropionitrile] in 9 ml of dry toluene under a nitrogen atmosphere was added 0.34 ml (1.3 mmol) of tributyltin hydride. The reaction mixture was heated at 85°C for 3.5 hours and an additional 0.17 ml of tributyltin  
10 hydride was added to the reaction mixture. The reaction mixture was stirred at 85°C for one hour and concentrated under vacuum at about 50°C. The residue was triturated twice with 15 ml of hexane. The hexane was decanted and the residue was concentrated under  
15 vacuum. The residue was chromatographed over silicon dioxide employing ethyl acetate/hexane (1.5/1, v/v). Fractions containing the major component were combined and the solvent was evaporated therefrom to provide 0.09  
20 g of  $\beta$ -1-(5-methyl-2,4-dioxo-1H,3H-pyrimidin-1-yl)-5-acetyl-2,3-dideoxy-2,2-difluororibose.

A solution of 0.09 (0.30 mmol) of the above intermediate was stirred in a saturated solution of anhydrous ammonia in methanol (10 ml) at 0-5°C for 2  
25 hours. The solution was evaporated to dryness under vacuum. The white residue was dissolved in a minimum amount of methanol and 20 ml of methylene chloride was added. This solution was stripped to 3 ml volume and more methylene chloride was added until crystals formed. The crystals were collected to yield 51 mg of

the desired product.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz)  $\delta$  1.85 (s, 3H, 5- $\text{CH}_3$ ); 2.5 (m, 2H, 2'); 3.65 (m, 1H, 5'A); 3.9 (m, 1H, 5'B); 4.3 (m, 1H, 4'); 6.1 (m, 1H, 1'); 7.8 (s, 1H, 6-H). Mass spec.  $m/e = 262 = P$ .

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Preparation 1

$\beta$ -1-(2,6-diamino-9H-purin-9-yl)-5-benzoyl-2-deoxy-2,2-difluororibose

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To a solution of 11.16 g (22 mmol) of  $\alpha$  &  $\beta$ -1-(2,6-diamino-9H-purin-9-yl)-3,5-dibenzoyl-2-deoxy-2,2-difluororibose was added hydrazine (0.7 g, 22 mmol). The solution was heated at 65°C for three hours. An additional amount of hydrazine was added (0.35 g, 11 mmol) and heating was continued for four hours. One additional amount of hydrazine (0.35 g, 11 mmol) was added and the solution was heated for four more hours. The reaction mixture was allowed to cool and the solvent was removed under reduced pressure. The resulting residue was put onto a silica gel column and eluted with a gradient from 2.5% methanol in methylene chloride to 5% methanol in methylene chloride. The remaining amount of starting material came off the column first followed by the alpha anomer (4.96 g) and then the beta anomer (1.75 g).  $^1\text{H}$  NMR ( $\text{DMSO } d_6$ , 300 MHz)  $\delta$  4.25 (m, 1H, 3'); 4.7 (m, 3H, 5' A & B, 4'); 5.95 (bs, 2H,  $\text{NH}_2$ ); 6.15 (m, 1H, 1'); 6.5 (d, 1H, OH); 6.85 (bs, 2H,  $\text{NH}_2$ ); 7.7 (m, 5H, Bz); 7.82 (s, 1H, C-8).

30 Mass spec. = 407 = P+1.



Example 6

$\alpha$  and  $\beta$ -1-(6-amino-9H-purin-9-yl)-2,3-dideoxy-2,2-difluororibose

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To a solution of 22.1 g (58 mmol) of 3,5-bis(benzoyl)-2-deoxy-2,2-difluororibose and 8.0 g (116 mmol) of imidazole in 410 ml of dimethylformamide (DMF) was added 8.8 g (58 mmol) of t-butyldimethylsilyl chloride. This reaction mixture was stirred for 12 hours at ambient temperature. The mixture was evaporated to dryness under reduced pressure. The residue was mixed with ethyl acetate and washed with 1.0N hydrochloric acid, saturated sodium bicarbonate, water, saturated sodium chloride solution, dried with sodium sulfate and evaporated to dryness under reduced pressure to yield 24.5 g of 1-(t-butyldimethylsilyloxy)-3,5-bis(benzoyl)-2-deoxy-2,2-difluororibose.

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To a solution of 19.12 g (39 mmol) of the above intermediate dissolved in methanol (560 ml) and cooled to  $-20^{\circ}\text{C}$  was added solid sodium methoxide (1.79 g, 33 mmol) portionwise while maintaining the reaction temperature at  $-20^{\circ}\text{C}$ . The reaction mixture was stirred at  $-20^{\circ}\text{C}$  for 3 hours at which time it was neutralized with acetic acid (2 g). The mixture was evaporated to a residue under reduced pressure at  $40^{\circ}\text{C}$ . The residue was mixed with water and ethyl acetate. The organic layer was separated and washed with water and saturated sodium chloride solution, dried with sodium sulfate and evaporated under reduced pressure. The resulting

residue was chromatographed on silica gel and eluted with 4:1 hexane:ethyl acetate. The product fractions were combined and evaporated under reduced pressure to product fractions were combined and evaporated under  
5 reduced pressure to yield 10.4 g of 1-(t-butyldimethylsilyloxy)-5-benzoyl-2-deoxy-2,2-difluororibose. Mass spec.  $m/e = 389 = P+1$ ,  $m/e = 331 = P - t\text{-butyl}$ .

To a solution of 10.4 g (27 mmol) of the above intermediate and 0.05 g of 4-dimethylamino-  
10 pyridine in 220 ml of dry pyridine under a nitrogen atmosphere was added 4.6 g (3.7 ml, 27 mmol) of phenyl chlorothionocarbonate. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum at 50°C to  
15 provide 14.2 g of 1-(t-butyldimethylsilyloxy)-3-(O-phenylcarbonothiooxy)-5-benzoyl-2-deoxy-2,2-difluororibose which was used without further workup. Mass spec.  $m/e = 467 = P - t\text{-butyl}$ .

To a solution of 14.24 g (27 mmol) of the above intermediate and 0.05 g of 2,2'-azobis[2-methyl-  
20 propionitrile] in 280 ml of dry toluene under a nitrogen atmosphere was added 14.32 ml (54 mmol) of tri-n-butyltin hydride. The reaction mixture was heated at 85°C for 4.5 hours. The reaction mixture was  
25 evaporated therefrom to provide 30.57 g of crude 1-(t-butyldimethylsilyloxy)-5-benzoyl-2,3-dideoxy-2,2-difluororibose which was used without further purification.  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  .15 (m, 6H,  $\text{SiCH}_3$ ); .95 (m, 9H, t-butyl); 2.45 (m, 2H, 3'A&B); 4.5  
30 (series of m, 3H, 4'&5'A&B); 5.2 (m, 1H, 1'); 7.8 (series of m, 5H, Bz).

To a solution of 1.0 g (2.7 mmol) of the above intermediate in 15 ml of tetrahydrofuran was added 5.4 ml (5.4 mmol) of a 1.0N solution of tetrabutyl ammonium fluoride in tetrahydrofuran. The reaction mixture was stirred at ambient temperature for 2 hours. The reaction mixture was evaporated under reduced pressure and the resulting residue was dissolved in ethyl acetate and washed with water, dried over sodium sulfate and evaporated under reduced pressure. The resulting oil was put on a silica gel column and eluted with a gradient from 5% ethyl acetate in hexane to 25% ethyl acetate in hexane. The product fractions were combined and evaporated under reduced pressure to yield 0.15 g of 5-benzoyl-2,3-dideoxy-2,2-difluororibose. Mass spec.  $m/e = 258 = P$ .

To a solution of 0.23 g (1.5 mmol) of 6-chloropurine in 10 ml of tetrahydrofuran was added 0.40 g (1.5 mmol) of triphenylphosphine and 0.26 g (1.5 mmol) of diethyl azodicarboxylate. To this solution was added a solution of 0.26 g (1.0 mmol) of the above intermediate in tetrahydrofuran. The reaction mixture was stirred at room temperature for approximately 12 hours. The solvent was evaporated under vacuum and the residue was chromatographed over silica and eluted with 2:1 hexane:ethyl acetate. Fractions containing the products were combined and the solvent was evaporated to provide 50 mg of  $\alpha\&\beta$ -1-(6-chloro-9H-purin-9-yl)-5-benzoyl-2,3-dideoxy-2,2-difluororibose. Mass spec.  $m/e = 395 = P + 1$ .

A solution of 50 mg (0.127 mmol) of the above mixture of intermediates dissolved in 9 ml of methanol was saturated with anhydrous ammonia at about 0°C. The reaction flask was sealed, and the mixture was allowed to warm to room temperature. The mixture was stirred for about 12 hours at room temperature and the volatiles were evaporated under reduced pressure to provide 40 mg of the desired products as a mixture. Mass spec.  $m/e = 271 = P$ .

10 The present invention provides a method of treating susceptible neoplasms in mammals comprising administering to a mammal in need of such treatment an antineoplastically effective amount of a compound of Formula I. The method comprises administering the compound to the mammal by various routes including the oral, rectal, transdermal, subcutaneous, intravenous, intramuscular or intranasal routes.

15 The term "antineoplastically effective amount", as defined herein, refers to an appropriate amount of a compound of Formula I which is capable of providing chemotherapy to mammals, especially humans. The active compounds are effective over a wide dosage range. For example, dosages per day will normally fall within the range of about 0.1 to about 1200 mg/kg of body weight. In the treatment of adult humans, the range of about 0.1 to about 50 mg/kg, in single or divided doses, is preferred. However, it will be understood that the amount of compound actually administered will be determined by a physician, in the light of the relevant circumstances including the condition

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to be treated, the particular compound to be administered, the chosen route of administration, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the  
5 above dosage ranges are not intended to limit the scope of the invention in any way.

The term "susceptible neoplasm", as defined herein, represents an abnormal growth of tissue in mammals capable of being treated by a compound of  
10 Formula I. While the compounds of Formula I are effective against tumors, both solid and non-solid type, the compounds are effective in controlling the growth of rapidly dividing cells because of the compounds' cytotoxic nature. Examples of tumors which the compounds  
15 of Formula I would be effective against include L1210V lymphocytic leukemia, 6C3HED, CA755, P1534J, X5563 Myeloma and the like.

The antineoplastic activity of a representative compound of the present invention has been demonstrated in a standard screen commonly used by those in  
20 the art in testing compounds for potential antitumor drugs. For example, these screens have been used to demonstrate the antitumor activity of commercially available cancer drugs such as the vinca alkaloids.  
25 See, e.g., Miller et al., in J. Med. Chem. Vol. 20, No. 3 409 (1977) and Sweeney, et al., in Cancer Research 38, 2886 (1978).

The compounds of the present invention represented by Formula I are cytostatic in that they  
30 inhibit the growth of human leukemic cells (CCRF-CEM

cell line). Table 1 below gives the results of such testing of compounds representative of those in Formula I. In the Table, column 1 gives the example number of the compound and column 2 the IC<sub>50</sub> (concentration giving 50% growth inhibition) in mcg/ml.

Table 1  
Cytotoxicity Screen

10	<u>Compound</u>	<u>IC<sub>50</sub></u> <u>(mcg/ml)</u>
	Example 1	1.9
	Example 2	1.9
15	Example 3	8.5
	Example 4	23.4
	Example 5	>20

The compounds of the present method are also effective for the treatment of viral infections, and more particularly in the treatment of infections caused by viruses of the Herpes genus. As such, yet another embodiment of the present invention is a method of treating viral infections in mammals comprising administering to a mammal in need of such treatment an antivirally effective amount of a compound of Formula I.

The term "antivirally effective amount", as defined herein, refers to an appropriate amount of a compound of Formula I which is capable of preventing or inhibiting the presence of viral infections in mammals. In general, dosage rates in the range of from about 5 mg/kg to about 500 mg/kg are useful. It is more preferred to administer at rates in the range of from

about 10 mg/kg to about 100 mg/kg.

The compounds defined by Formula I can be employed to treat or prevent diseases commonly caused by a wide range of viruses. Typical viruses against  
5 which the compounds of the invention can be used to control include all A and B strains of influenza, para-influenza, respiratory syncytial viruses, the various Herpes I and Herpes II strains, Echo and  
10 vaccinia viruses, measles, Semliki Forest and retroviruses such as Friend's Leukemia Virus and those responsible for causing acquired immune deficiency syndrome.

The following plaque-reduction study provides a quantitative evaluation of inhibitors of virus multi-  
15 plication and establishes the antiviral activity of a representative compound of the present invention.

According to this study, susceptible cells (BSC-1, Hela, MDCK, etc.) were grown in 25 cm<sup>2</sup> Falcon  
20 flasks at 37°C in Medium 199 with 5 percent inactivated fetal bovine serum (FBS), penicillin (150 units/ml) and streptomycin (150 µg/ml). When confluent monolayers were formed, the growth medium was removed and 0.3 ml of an appropriate dilution of the virus was added to each flask. After adsorption for one hour at room tempera-  
25 ture, the infected cell sheet was overlaid with equal parts of 1 percent Agarose and 2x Medium 199, 2.5 percent FBS, penicillin, and streptomycin. The compound to be tested was dissolved in dimethyl sulfoxide (DMSO)

at a concentration of 10,000 µg/ml and an aliquot of the resulting solution was diluted to the desired concentration with the agar medium mixture. Flasks were incubated at 37°C until control flasks indicated optimum plaque size of about 2 to about 10 mm. A solution containing 10 percent formalin by volume and 2 percent sodium acetate by volume was added to each flask to inactivate the virus and fix the cell sheet to the plastic surface. The plaques were counted after staining the surrounding cell areas with crystal violet. Results from duplicate flasks at each concentration were averaged and compared with control flasks. The following degrees of inhibition against HSV-1 were observed.

Compound	Concentration	Inhibition
Ex. 1	1.0 µg/ml	50%
Ex. 2	3.1	27%
Ex. 4	25	39%
Ex. 5	25	35%

The following degrees of inhibition against HSV-2 were observed.

Compound	Concentration	Inhibition
Ex. 3	25 µg/ml	21%
Ex. 4	13.5	50%



The following procedure was used to establish the efficacy of a representative compound of the present invention against Friends leukemia virus.

5 SC-1 cells from a feral mouse were seeded subconfluently at  $2-5 \times 10^3$  cells per well in 96 micro-  
well plates with complete MEM media plus 2 mcg/ml  
polybrene overnight at  $37^\circ\text{C}$  under a carbon dioxide  
atmosphere. The medium was removed. The cultures  
were infected with a proper dilution of murine leukemia  
10 virus (50 mcl/well) and were allowed to adsorb for  
about two hours at room temperature. After adsorption,  
the virus containing medium was removed and replaced  
with fresh complete MEM, both with and without dilutions  
of the compound of Example 1, and reincubated at  $37^\circ\text{C}$   
15 under carbon dioxide for 5 days or until the cells  
reached confluence. The medium was removed and the  
cells were exposed for 10 seconds to a UV germicidal  
light. XC cells from a Rous sarcoma virus induced rat  
tumor cell line at a concentration of  $5-8 \times 10^4$  cells/  
20 well were added to the irradiated SC-1 monolayers.  
The cultures were incubated at  $37^\circ\text{C}$  under carbon dioxide  
for three days until CPE occurred in the control wells.  
Cultures were fixed with formalin and stained with  
crystal violet. The results were scored as CPE inhibi-  
25 tion. The compound of Example 1 had an  $\text{IC}_{50}$  of 6.5  
 $\mu\text{g/ml}$ .

The compounds of the present invention are preferably administered as a pharmaceutical formulation. Therefore, as yet another embodiment of the present  
30 invention, a pharmaceutical formulation useful for  
treating susceptible neoplasms in mammals is provided

comprising a compound of Formula I in combination with a pharmaceutical carrier, diluent or excipient therefor.

The active ingredient will be present in the formulation in the range of about 1% to about 90% by weight. The active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents

or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained release of the active ingredient after administration to the patient by employing procedures well known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 500 mg, more usually about 25 to about 300 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

The following formulation examples represent specific pharmaceutical formulations employing compounds comprehended by the present method. The formulations may employ as active compounds any of the compounds of Formula I. The examples are illustrative only and are not intended to limit the scope of the invention in any way.

#### Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	<u>Quantity (mg/capsule)</u>
1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-3-amino-2,2-difluororibose	250
5 starch dried	200
magnesium stearate	10

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

#### Formulation 2

A tablet formula is prepared using the ingredients below:

	<u>Quantity (mg/tablet)</u>
1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2,3,3-tetrafluororibose	250
cellulose, microcrystalline	400
20 silicon dioxide, fumed	10
stearic acid	5

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

An aerosol solution is prepared containing the following components:

	<u>Weight %</u>
5	
1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluoro-ribose	0.25
ethanol	29.75
10	
Propellant 22 (chlorodifluoromethane)	70.00

The active compound is mixed with ethanol and the mixture added to a portion of the Propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then placed in a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

Tablets each containing 60 mg of active ingredient are made up as follows:

25	1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluoro-ribose	60 mg
	starch	45 mg
	microcrystalline cellulose	35 mg
30	polyvinylpyrrolidone (as 10% solution in water)	4 mg
	sodium carboxymethyl starch	4.5 mg
	magnesium stearate	0.5 mg
	talc	1 mg

5 The difluoronucleoside, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

#### Formulation 5

15 Capsules each containing 80 mg of medicament are made as follows:

20	1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-3-azido-2,2-difluoroxylene	80 mg
	starch	59 mg
	microcrystalline cellulose	59 mg
	magnesium stearate	2 mg

25 The active ingredient, cellulose, starch and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

Suppositories each containing 225 mg of difluoronucleoside are made as follows:

5	1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose	225 mg
	saturated fatty acid glycerides to	2 g

10

The nucleoside is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

15

Formulation 7

Suspensions each containing 50 mg of medication per 5 ml dose are made as follows:

20	1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose	50 mg
	sodium carboxymethyl cellulose	50 mg
25	syrup	1.25 ml
	benzoic acid solution	0.10 ml
	flavor	q.v.
	color	q.v.
30	purified water to	5 ml

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation 8

10 An intravenous formulation is prepared as follows:

	1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2,3-dideoxy-2,2-difluororibose	100 mg
15	isotonic saline	1000 ml

The solution of the above ingredients is administered intravenously at a rate of 1 ml/minute to a mammal in need of treatment from susceptible neoplasms.

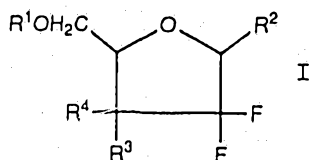


The claims defining the invention are as follows:

~~What we claim is:~~

1. A compound of the formula

5



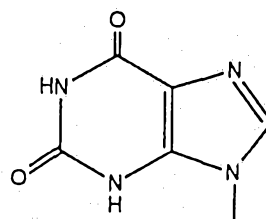
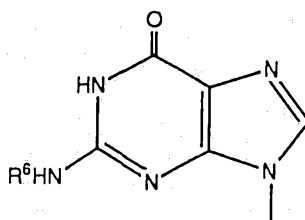
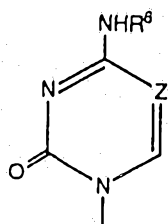
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wherein:

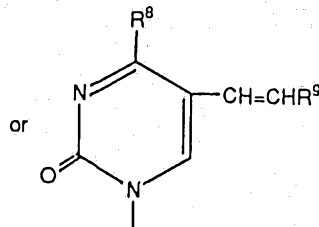
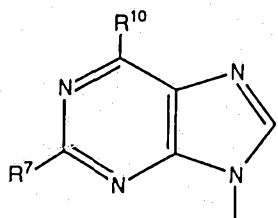
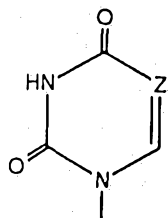
15

$R^1$  is hydrogen,  $C_1$ - $C_4$  alkyl or  $-C(=O)-R^5$ ;  
 $R^2$  is a base defined by one of the formulae

20



25



30

R<sup>3</sup> is hydrogen, amino, azido or fluoro;  
R<sup>4</sup> is hydrogen or fluoro;  
each R<sup>5</sup> independently is hydrogen or C<sub>1</sub>-C<sub>4</sub>

alkyl;

5

R<sup>6</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl or  $\begin{array}{c} \text{O} \\ || \\ -\text{C}-\text{R}^5 \end{array}$ ;

10

R<sup>7</sup> is hydrogen, C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> haloalkyl,  
amino, bromo, fluoro, chloro or iodo;

R<sup>8</sup> is hydroxy or amino;

R<sup>9</sup> is hydrogen, bromo, chloro or iodo;

R<sup>10</sup> is -NHR<sup>6</sup>, bromo, chloro, hydroxy, fluoro  
or iodo;

15

Z is N or C-R<sup>7</sup>; or

a pharmaceutically-acceptable salt thereof;  
with the proviso that when R<sup>4</sup> is fluoro, R<sup>3</sup>  
is other than amino or azido.

20

2. A compound of Claim 1 wherein R<sup>3</sup> and R<sup>4</sup>  
are independently fluoro or hydrogen.

3. A compound of Claim 1 wherein R<sup>3</sup> is amino  
or azido.

25

4.  $\beta$ -1-(4-Amino-2-oxo-1H-pyrimidin-1-yl)-  
2,3-dideoxy-2,2-difluororibose or a pharmaceutically-  
acceptable salt thereof.

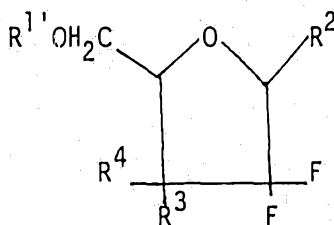
5.  $\beta$ -1-(2,6-Diamino-9H-purin-9-yl)-2,3-di-  
deoxy-2,2-difluororibose or a pharmaceutically-acceptable  
salt thereof.

30

6. A pharmaceutical formulation comprising  
as an active agent a compound of any one of Claims 1 to  
5, associated with one or more carriers, diluents or  
excipients therefor.



7. A process for preparing a compound of formula I wherein  $R^1$  is hydrogen comprising (A) deblocking a compound of the formula



wherein  $R^1$  is a hydroxy-protecting group; or (B) reacting a compound of Formula I wherein  $R^1$  is hydrogen with an acidic salt-forming reagent.

8. A compound of Formula I wherein  $R^1$  is hydrogen whenever prepared by the process of claim 7.

9. A 2',3'-dideoxy-2',2'-difluoronucleoside, as herein described with reference to the final product of Example 1, or any one of Examples 2 to 6.

10. A process for preparing a 2',3'-dideoxy-2',2'-difluoronucleoside, substantially as herein described with reference to the preparation of the final product of Example 1, or any one of Examples 2 to 6.

11. A pharmaceutical formulation comprising a compound of any one of claims 1 to 9 together with a pharmaceutically acceptable carrier, diluent and/or excipient.

12. A pharmaceutical formulation comprising a 2',3'-dideoxy-2',2'-difluoronucleoside together with a pharmaceutically acceptable carrier, diluent and/or excipient which formulation is substantially as herein described with reference to any one of Formulations 1, 3, 4, 5, 6, 7 or 8.

13. A method of treating susceptible neoplasma in a mammal requiring such treatment, comprising administering to said mammal an antineoplastically effective amount of a compound of any one of claims 1 to 5, 8 or 9 and/or a pharmaceutical formulation of any one of claims 6, 11 or 12.



14. A method of treating viral infections in a mammal requiring such treatment, comprising administering to said mammal an antivirally effective amount of a compound of any one of claims 1 to 5, 8 or 9 and/or a pharmaceutical formulation of any one of claims 6, 11 or 12.

DATED this THIRD day of DECEMBER 1990  
Eli Lilly and Company

Patent Attorneys for the Applicant  
SPRUSON & FERGUSON

