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Bardos et al.

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[54] **5-IOSEO-2-PYRIMIDINONE NUCLEOSIDE**

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Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 641,770, Aug. 20,
1984, Pat. No. 4,782,142, which is a continuation-in-
part of Ser. No. 337,297, Jan. 5, 1982, Pat. No.
4,468,384.

[51] Int. Cl.⁴ **C07H 19/067**

[52] U.S. Cl. **536/23**

[58] Field of Search **536/23; 514/49**

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[57] **ABSTRACT**

The nucleoside 1-(2-Deoxy- β -D-ribofuranosyl)-5-
(iodo)-2-pyrimidinone possesses a high level of antiviral
activity and a low level of toxicity to the host cell mak-
ing it an especially effective therapeutic agent for HSV-
2.

1 Claim, No Drawings

5-ISO-2-PYRIMIDINONE NUCLEOSIDE

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of application Ser. No. 641,770, filed Aug. 20, 1984, now U.S. Pat. No. 4,782,142 which is a continuation-in-part of application Ser. No. 337,297, filed Jan. 5, 1982, now U.S. Pat. No. 4,468,384.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to novel compounds for inhibiting the replication of DNA viruses, and more particularly, relates to a compound for inhibiting the replication of DNA viruses which induce the formation of thymidine kinase enzyme.

2. History of the Prior Art

Historically, viruses have been the causative agents of many diseases of both plants and animals including man. Diseases caused by viruses have been very difficult to control or cure by traditional methods. Many such viral diseases have been, in the past, effectively controlled through mass vaccination but even in modern times, effective agents to cure viral diseases, rather than prevent them, have been unavailable.

It has been recently discovered that certain substituted naturally occurring pyrimidinones are effective antiviral agents. Most of such compounds are 5-substituted pyrimidinones attached to a pentose sugar group at the one position of the pyrimidinone ring. Examples of such compounds and their effects are discussed in "Molecular Basis for Serendipitous Development of Antiviral and Anticancer Aminonucleosides" by W. H. Prusoff et al; "Comparative Study of the Potency and Selectivity of Anti-Herpes Compounds" by DeClercq and "Strategy for the Development of Selective Anti-Herpes Virus Agents Based on the Unique Properties of Viral Induced Enzymes—Thymidine Kinase, DNase and DNA Polymerase". All of these articles appear in Volume 57 of a Symposium of the Federation of European Biochemical Societies, Antimetabolites in Biochemistry, Biology and Medicine edited by Skoda et al, published by Pergamon Press (1978).

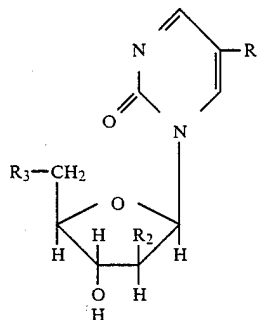
Unfortunately, such antiviral compounds, based upon naturally occurring pyrimidinones have a serious disadvantage in that these compounds are rapidly metabolized, generally having a metabolic half life of less than 30 minutes. Such short metabolic life has not permitted such compounds to be effectively used under In Vivo conditions.

Certain compounds, based upon 4-Deoxy uracil have recently been synthesized by two of the inventors herein and presented in a thesis by Alan Curtis Schroeder in 1978. Such thesis does not in general discuss or suggest any anti-viral activity by 5 substituted 4-Deoxy uracil compounds except on page 98 of the thesis wherein it was indicated that such compounds would be tested against Herpes Virus in mouse L cells. There was no indication that such compounds would in fact have any effect after such tests.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the invention, there is provided a method for inhibiting the replication of the DNA virus which induces formation of thymidine kinase enzyme.

In accordance with the method, the virus is exposed to an effective concentration of the compound of the formula:

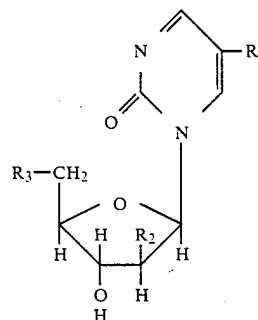


wherein R_1 is a radical selected from the group consisting of halogen, $-SCH_3$, $-OH$, alkoxy, cyano, methylamino, carboxy, formyl, nitro and unsubstituted or halosubstituted hydrocarbon groups of 1 through 3 carbon atoms; R_2 is hydrogen or hydroxy; and R_3 is hydroxy, $-OP(O)(OH)_2$, amino, or $-OCOR_4$ where R_4 is lower alkyl of 1 through 6 carbon atoms.

In furtherance of the invention, anti-viral compounds are provided which include those where R_1 of the above formula is a radical selected from the group of chloro, iodo, hydroxy, alkoxyalkyl, hydroxyalkyl, methylamino, formyl, nitro, unsubstituted hydrocarbon groups of 2 to about 3 carbon atoms or halosubstituted hydrocarbon groups of 1 to about 3 carbon atoms; R_2 is hydrogen or hydroxy, and R_3 is hydroxy, $-OP(O)(OH)_2$, amino or $-OCOR_4$ where R_4 is alkyl, alkenyl or alkoxyalkyl of 2 to about 18 carbon atoms.

DETAILED DESCRIPTION OF THE INVENTION

As previously discussed, the method of the invention comprises inhibiting the replication of a DNA virus which induces formation of thymidine kinase enzyme, by exposing the virus to an effective concentration of a compound of the formula:



In accordance with the methods of the invention, R_1 of the formula is a radical selected from the group consisting of halogen, $-SCH_3$, $-OH$, alkoxy, cyano, methylamino, carboxy, formyl, nitro and unsubstituted or halosubstituted hydrocarbon groups of 1 through 3 carbon atoms. The most preferred R_1 groups are halogen, alkyl, haloalkyl, alkenyl, haloalkenyl, alkynyl or haloalkynyl groups. Particular compounds which will

have good effectiveness are those compounds wherein R_1 is selected from methyl, ethynyl, ethyl, propyl, propynyl, iodo and bromo groups. Such compounds have been unexpectedly found to have superior effectiveness over those compounds wherein R_1 is an iodo or ethynyl group.

As previously discussed, R_2 is hydrogen; halogen or hydroxy but is preferably hydrogen, fluorine or hydroxy. R_3 , as previously discussed is hydroxy, $-\text{OP}(\text{O})(\text{OH})_2$, amino or $-\text{OCOR}_4$ where R_4 is lower alkyl of 1 through 6 carbon atoms. Preferably, R_3 is hydroxy. Compounds wherein R_3 is $\text{OP}(\text{O})(\text{OH})_2$, amino or $-\text{OCOR}_4$, as previously discussed, are generally in themselves, not effective but in In Vivo environments are rapidly converted to compounds wherein R_3 is hydroxy which then undergo further metabolic activation.

As indicated above in connection with the compounds of the present invention R_1 includes various radicals like alkoxyalkyl and hydroxyalkyl. Specific representative examples include, but are not limited to, methoxymethyl, methoxyethyl, hydroxymethyl, and 2-hydroxypropyl. In addition to these radicals the compounds as disclosed hereinabove include unsubstituted hydrocarbon groups of 2 to about 3 carbon atoms or halosubstituted hydrocarbons having from 1 to about 3 carbon atoms. Typical preferred examples of such groups include ethyl, propyl, ethynyl, propynyl, and bromo-vinyl, to name but a few.

Besides hydroxy and $-\text{OP}(\text{O})(\text{OH})_2$ groups for R_3 , the compounds of the present invention include esters designated by OCOR_4 . R_4 may be an alkyl, alkenyl or an alkoxyalkyl radical having from 2 to about 18 carbon atoms, and include, for example, preferred members like butyl, undecyl and heptadecenyl and their isomers, such as iso-butyl, sec.-butyl, and t-butyl.

Examples of compounds suitable for use in accordance with the invention are:

1. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone
2. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(methyl)-2-pyrimidinone
3. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(iodo)-2-pyrimidinone
4. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(bromo)-2-pyrimidinone
5. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(trifluoromethyl)-2-pyrimidinone
6. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(nitro)-2-pyrimidinone
7. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(cyano)-2-pyrimidinone
8. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(ethynyl)-2-pyrimidinone
9. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(propynyl)-2-pyrimidinone
10. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(propyl)-2-pyrimidinone
11. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(bromo-vinyl)-2-pyrimidinone
12. 1-(2-Deoxy- β -D-ribofuranosyl)-5-(formyl)-2-pyrimidinone
13. 1-(2-Deoxy, 2-fluoro- β -D-arabinofuranosyl)-5-(methyl)-2-pyrimidinone
14. 1-(β -D-arabinofuranosyl)-5-(methyl)-2-pyrimidinone

Two especially effective compounds for use in accordance with the present invention is the compound

wherein R_1 is iodo or ethynyl; R_2 is hydrogen and R_3 is hydroxy.

The preferred deoxyriboside of the present invention is where R_1 is iodo. This particular compound has a high level of anti-viral activity, particularly against herpes virus group and a low level of toxicity for the host cell. In relation to the 1-(2-Deoxy- β -D-ribofuranosyl)-5-(iodo)-2-pyrimidinone, Oftebro et al., *Biochemical Pharmacology*, Vol. 21, Pergamon Press (1972) pp. 2451-2456 and Oyen et al., *Biochim Biophys. Acta*, 182 (1969) pp. 567-569 each disclose the 5-fluoro derivative of 1-(2-Deoxy- β -D-ribofuranosyl)-2-pyrimidinone. Although exhibiting anti-viral activity, the structurally related fluoro substituted analogue was found to be more toxic to the host cell than to the virus, and therefore, was found not to have any potential value as an anti-viral agent against HSV-2. Accordingly, the 5-iodo-pyrimidin-2-one-deoxyriboside and its combined properties were indeed an important and surprising discovery.

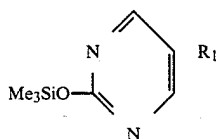
Another 5-iodo deoxyriboside has been successfully used in treating Herpes virus. Torrence et al., *Frontiers in Bioorganic Chemistry And Molecular Biology*, Elsevier/North-Holland, Biomedical Press (1979) pp. 59-85 disclose 5-iodo-2'-deoxyuridine widely known by the generic name idoxuridine. Although known to be useful against Herpes virus, idoxuridine is rapidly inactivated by nucleotidases upon systemic administration to a host species. After intravenous injection, most of the active form of the drug disappears from the blood in about thirty minutes. In addition, it has been reported that the systemic administration of idoxuridine may produce toxicities, such as bone marrow depression. Consequently, therapeutic use of idoxuridine is limited almost exclusively to topical administration. At present, idoxuridine is limited to topical treatment of herpes simplex keratitis of the eye. It has been reported that herpatic lesions of the skin do not respond. Hence, idoxuridine has very limited applications even when employed topically. In contradistinction, 5-iodo-pyrimidin-2-one-deoxyriboside of the present invention can be used against HSV-2 and can be administered orally, intraperitoneally or subcutaneously without significant toxicity to the host or loss of activity as demonstrated by Example 9 below.

5-halo-pyrimidin-2-one ribosides have been reported in the literature. For example, East German Patent 134,429 (1979) discloses a single compound, namely 5-fluoro-pyrimidin-2-one riboside as possessing anti-viral activity. However, as pointed out above, the more closely structurally related 5-fluoro deoxyriboside was found to possess anti-viral activity, but was more toxic to the host than to the virus as demonstrated in Example 8 below.

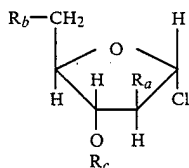
McCormick et al., *Biochemical Pharmacology*, Vol. 29 (1980) pp. 830-832 reported on the 5-fluoro, 5-chloro and 5-bromo derivatives of pyrimidine-2-one 1- β -D-ribofuranosyl. McCormick et al. reported that the 5-fluoro and 5-bromo derivatives show similar activities as inhibitors of the enzyme cytidine deaminase. But, when tested for anti-tumor activity against murine leukemia L 1210 only the 5-fluoro was active. Hence, while McCormick et al. seem to show similarities in activity between fluoro, chloro and bromo derivatives of 1- β -D-ribofuranosyl in the inhibition of cytidine deaminase the lack of anti-tumor activity for the 5-chloro and 5-bromo substituted compounds fail to support the conclusion that the halogens are interchangeable in this

compound. McCormick et al. also failed to teach anti-viral activity or the 5-iodo homolog. The disclosure of cytidine deaminase inhibition or the limited anti-tumor activity of the 5-fluoro riboside is not viewed as a suggestion of possible anti-viral activity with respect to the 5-iodo-pyrimidine-2-one-deoxyriboside.

In general, such compounds are prepared by reacting a compound of the formula:



with a substituted sugar of the formula:



wherein R_a , R_b and R_c are radicals which are non-reactive during the reaction of I with II and which can be converted to the desired R_2 , R_3 and H respectively after reaction of I with II. Detailed discussions of how compounds for use in accordance with the method of the present invention can be prepared are found in Synthesis and Antiviral Activity of 1-(2-Deoxy- β -D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone by Schroeder et al published in the Journal of Medicinal Chemistry, Volume 24, No. 1, pages 109-112, available to the public Jan. 5, 1981. Further discussion of methods of synthesis of compounds for use in accordance with the method of the present invention is made by Wightman et al., Collection of Czechoslovakian Chemical Communications, Volume 38, beginning at page 1381 (1973) and "Synthesis of New Nucleoside Analogs Derived from 4-Deoxy uracil and 6-substituted Uracils" by Schroeder dissertation at the State University of New York at Buffalo (1978).

In accordance with the method of the invention, the replication of numerous viruses can be inhibited. In particular, viruses which induce the formation of thymidine kinase enzyme are inhibited in accordance with the method of the invention. Such viruses generally include essentially all Herpes type viruses including Herpes simplex type 1, Herpes simplex type 2, varicella zoster, Epstein-bar virus, Cytomegalo virus, varicella zoster, Herpes zoster and variola. It is known that such viruses cause numerous infections in man including localized infections such as infections of the eye and genitals.

The following examples serve to illustrate and not limit the present invention.

EXAMPLE 1

1-(2-Deoxy- β -D-Ribofuranosyl)-5-Ethynyl-2-Pyrimidinone

A suspension of 15.06 g (67.83 mmol) of 5-iodo-2(1H)-pyrimidinone was refluxed in 40 ml of hexamethyldisilazane and 2 ml of chlorotrimethylsilane for 1 hour. The excess reagent was removed in vacuo to yield a yellow oil 5-iodo-2-trimethylsilyloxy pyrimidine, which was dissolved with 30 ml of dry degassed trieth-

ylamine. To this solution was added 11.5 ml (81.37 mmol) of trimethylsilylacetylene, 0.32 g (2.5 mol %) of CuI and 0.48 g (1 mol %) of $(\phi_3P)_2PdCl_2$. The reaction mixture was stirred under nitrogen, at room temperature, for 3 days and diluted with 100 ml of dry THF. The mixture was filtered under dry nitrogen; the precipitate washed several times with dry THF and the filtrate was concentrated to a residue. The latter was treated with methanol to give a white crystalline, chromatographically homogeneous material 5-(2-Trimethylsilyl)ethynyl-2(1H)-pyrimidinone (9.99 g, 76.6%). Analytically pure material could be obtained by recrystallization from methanol; mp 203°-206° C.

1.41 g (7.32 mmol) of 5-(2-trimethylsilylethynyl)-2(1H)-pyrimidinone was refluxed in 14 ml of hexamethyldisilazane and 0.3 ml of chlorotrimethylsilane for 3 hours, and the resulting solution was concentrated to an oily residue. The residual solvent was removed by co-evaporation with 1,2-dichloroethane (2×20 ml). The residue was dissolved in 30 ml of anhydrous dichloroethane and the solution was added to a cooled solution of 2.5 g (5.82 mmol) of the halogenose 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy- α -D-ribofuranosyl)chloride. The cooled mixture was then treated, dropwise, with a solution of 0.17 ml of $SnCl_4$ in 25 ml of anhydrous 1,2-dichloroethane. The mixture was stirred at 0° C. for 2 hours and periodically monitored by tlc (15% ethyl acetate in methylene chloride; Macherey-Nagel silica gel G). The mixture was diluted with dichloroethane (100 ml) and saturated bicarbonate solution (50 ml), and filtered over Celite, the residue being washed several times with dichloroethane. The organic layer was subsequently washed with water (50 ml), dried over $MgSO_4$, and concentrated to a residue which was chromatographed on 100 g of silica gel (eluted with a gradient of 0 to 12% ethyl acetate in methylene chloride). The fractions containing the slower moving alpha anomer, 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy- α -D-ribofuranosyl)-5-(2-trimethylsilyl)ethynyl-2-pyrimidinone, were pooled, concentrated to a white residue which was recrystallized from ethanol to provide, after drying, 0.48 g (14.2%); mp 150°-152° C.

The faster moving beta anomer, 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy- β -D-ribofuranosyl)-5-(2-trimethylsilyl)ethynyl-2-pyrimidinone, was obtained in a similar manner to that described for the alpha anomer. After recrystallization from ethanol and drying, 0.42 g (12.4%) of the white powder was obtained; mp 183°-185° C.

0.31 g (0.5 mmol) of the beta anomer was then added to 20 ml of cold anhydrous methanol presaturated with dry ammonia, and the mixture, after sealing the reaction vessel, was stirred at 4° C. for 6 hours. The resulting solution was concentrated to an oil; the latter was treated with acetone and concentrated to a minimum volume with concomitant precipitation of the product. The mixture was treated with small amounts of acetone-ether and cooled. The product, 1-(2-deoxy- β -D-ribofuranosyl)-5-ethynyl-2-pyrimidinone, was collected by filtration, washed with acetone-ether and dried to yield 69 mg (55.9%) of the off-white powder; dec above 130° C.; NMR ($DMSO-d_6 + D_2O$) δ 2.26 (m, 2, 2'-H), 3.68 (m, 2, 5'-H), 3.97 (m, 1, 4'-H), 4.27 (m, 2, 3'-H and C-H), 6.03 (t, 1, 1'-H); $J_{1,2} = 6$ Hz), 8.63 (d, 1, 6-H, $J_{6,4} = 3$ Hz), 8.73 (d, 1, 4-H, $H_{4,6} = 3$ Hz); IR (KBr) ν max 3525 (shoulder), 3220 (broad), 2925 (shoulder), 2100, 1660, 1423, 1350, 1265, 1110, 1070 (shoulder), 925

cm⁻¹; UV (MeOH) lambda max 32 nm Anal. (C₁₁H₁₂N₂O₄); C, H, N.

EXAMPLE 2

1-(2-Deoxy-β-D-Ribofuranosyl)-5-(1-propynyl)-2-pyrimidinone

31.67 g (0.13 mol) of 5-iodo-2-methoxypyrimidine, 0.73 g (2.9 mol %) of CuI and 1.02 g (1.1 mol%) of bis(triphenylphosphine)PdCl₂ were suspended in 200 ml of anhydrous triethylamine (distilled over BaO) in Parr pressure bottle. The bottle was evacuated and filled with 85% propyne. The bottle was repeatedly shaken and filled with propyne until the pressure had stabilized at 20 psi, and then the mixture was shaken, in a Parr hydrogenator, for 24 hours. More propyne was added at this time shaking was continued for an additional 48 hours. On tlc (methylene chloride), there was only one major product; no starting material was observed. The mixture was diluted with methylene chloride (300 ml), washed with saturated bicarbonate solution (2×60 ml), dried over magnesium sulfate and concentrated to a minimum volume with attendant precipitation. Petroleum ether was added to the mixture and after cooling, the first crop was obtained by filtration. The filtrate was concentrated to give a second crop. Both crops were dried and sublimed at 0.3 mm Hg (max. oil bath temp. 100° C.) to yield 15.26 g (76.7%) of analytically pure, white-crystalline material, 2-methoxy-5-(1-propynyl)-pyrimidine; mp 88°-89.5° C.).

Chlorotrimethylsilane (4.11 ml; 32.5 mmol) was introduced by syringe, into a solution of 1.5 g (10.2 mmol) of the 2-methoxy-5-(1-propynyl)pyrimidine and 4.56 g (30.44 mmol) of NaI in 35 ml of anhydrous acetonitrile maintained under an atmosphere of dry nitrogen and removed from light. The mixture was stirred at 50° C. for 2½ hours with periodic monitoring by tlc. The mixture was cooled in an ice-bath and 3.5 g (8.14 mmol) of the halogenose, 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl)-chloride were then added. To this mixture was added 3 ml of 1N trimethylsilyl triflate in 1,2-dichloroethane; the mixture was stirred at 0° C. for 1 hour and then at room temperature for 2½ hours. The reaction mixture was then diluted with dichloromethane (150 ml) and saturated aqueous bicarbonate (65 ml). The organic extract was subsequently washed with water (60 ml), dried over magnesium sulfate and concentrated to a residue which was subjected to flash chromatography on 80 g of silica gel. The fractions containing the slower moving alpha anomer were pooled and concentrated to a minimum volume with attendant precipitation. The mixture was cooled and filtered; the white product washed with diethyl ether and dried at 50° C. to provide 1.1 g (25.7%) of 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl)-5-(1-propynyl)-2-pyrimidinone; mp 176°-178° C.

The fractions containing the faster moving beta anomer 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl)-5-(1-propynyl)-2-pyrimidinone were pooled and concentrated to yield a foam which was subsequently dried.

300 mg (0.57 mmol) of the beta anomer was added to a cold, anhydrous, saturated solution of methanolic ammonia (15 ml), and the flask was sealed. The mixture was stirred at 4° C. for 4 hours. Tlc (10% MeOH/CH₂Cl₂) showed several minor fluorescent products. The solution was concentrated, in vacuo, to an oil which was chromatographed on 20×20 cm silica gel plates (10% MeOH/CH₂Cl₂). The major fluores-

cent band was extracted with a solution of 20% methanol in methylene chloride and the extract was concentrated to a syrup. The latter was dried to give 1-(2-deoxy-β-D-ribofuranosyl)-5-(1-propynyl)-2-pyrimidinone as a pale yellow, chromatographically homogeneous foam (82 mg; 57.7%); NMR (DMSO-d₆+D₂O) delta 1.18 (t, ethanol, H₂O), 1.83-2.67 (m, 5, H'-2 and -C≡CH₃), 3.3-4.5 (ethanol, H₂O and sugar protons), 6.08 (t, 1, H'-1, J_{1',2'}=6 Hz), 7.97 (dd, 2, H-4 and H-6; J_{4,6}=3 Hz); IR (KBr) Nu max 3350 (broad), 2920 (shoulder), 2600 (shoulder), 1650, 1500, 1250, 1090 cm⁻¹; Anal. (C₁₂H₁₄N₂O₄·0.4H₂O) C, H, N,

EXAMPLE 3

1-(2-Deoxy-β-D-Ribofuranosyl)-5-iodo-2-pyrimidinone

2-Pyrimidinone (6 g, 62.44 mmol) and N-iodosuccinimide (14.7 g, 65.3 mmol) in dry DMF (30 ml) were stirred for 48 hours at room temperature with the exclusion of both light and moisture. The mixture was added to ether (50 ml) with stirring and the supernatant was decanted. The precipitate was collected by filtration, repeatedly washed with acetone and finally with methanol, until the filtrate became light yellow. After drying in vacuo the yellow granular product, 5-iodo-2(1H)-pyrimidinone, appeared to be pure by tlc and spectra, but the elemental analysis indicated that it was contaminated with a trace amount of DMF. Yield: 11.35 g (77.5%).

A suspension of 0.86 g (3.87 mmol) of the 5-iodo-2(1H)-pyrimidinone in 15 ml of hexamethyldisilazane and 0.3 ml of chlorotrimethylsilane was refluxed for 2 hours (with the exclusion of moisture), cooled and concentrated in vacuo to yield a yellow oil. Residual solvent was removed by coevaporation with 1,2-dichloroethane (2×10 ml). 1.65 g (3.84 mmol) of the sugar halide 1-(3,5-di-O-(p-chlorobenzoyl)-2-(deoxy-α-D-ribofuranosyl) chloride, were added to the oil; the mixture was dissolved in 50 ml of anhydrous 1,2-dichloroethane and cooled in an ice bath. To the cooled solution was added, dropwise, a solution of 0.2 ml (1.7 mmol) of SnCl₄ in 25 ml of anhydrous dichloroethane. The reaction mixture was stirred at 0° C. for 2½ hours at which time tlc (17% ethyl acetate in dichloroethane) showed disappearance of the starting material, indicating that the reaction was essentially complete. The mixture was diluted with 60 ml of dichloroethane and 50 ml of saturated bicarbonate, and the resulting emulsion was filtered over Celite, the precipitate being washed several times with 1,2-dichloroethane. The organic layer was washed with 50 ml of water, dried over anhydrous magnesium sulfate and concentrated to a residue which on tlc showed two major spots of equal intensity (R_f values 0.19 for the alpha anomer, 1-[3,5-di-O-(p-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl]-5-iodo-2-pyrimidinone, and 0.3 for the beta anomer 1-[3,5-di-O-(p-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-5-iodo-2-pyrimidinone; 17% ethyl acetate dichloroethane; Macherey-Nagel silica gel G). This residue was chromatographed on 90 g of silica el and eluted with a gradient of 0 to 25% ethyl acetate in dichloroethane. The fractions, containing the slower moving alpha anomer were pooled, concentrated to a residue which weighed, after drying, 0.48 g (20.4%). The white crystalline compound was obtained by recrystallization from ethanol-acetone; mp 175°-176° C.; [α]_D²⁶=+20.03° (CHCl₃, c.0.108); NMR (CDCl₃) delta 2.8 (m, 2, 2'-H), 4.6 (d, 2, 5=H), 5.0 (t, 1, 4'-H), 5.65 (d, 1, 3'-H), 6.27 (d, 1, 1'-H,

$J_{1',2'}=7$ Hz), 7.25–8.12 (m, 8, phenyl), 8.18 (d, 1, 6-H, $J_{6,4}=3$ Hz), 8.68 (d, 1, 4-H, $J_{4,6}=3$ Hz); IR (CHCl₃) Nu max 3000, 1725, 1665, 1595, 1490, 1380, 1260, 1090, 1010 cm⁻¹; UV (CHCl₃) lambda max (epsilon max) 340 nm (3600). Anal. (C₂₃H₁₇Cl₂IN₂O₆) C, H, Cl, I, N.

The faster moving beta anomer was obtained in the same manner described for the alpha anomer to yield 0.46 g (19.5%) of 1-(3,5-di-O-(p-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl)-5-iodo-2-pyrimidinone; mp 136°–138° C.; $[\alpha]_D^{26}=-13.66\alpha$ (CHCl₃, c 0.10); ¹H NMR (CDCl₃) delta 2.32 (m, 1, 2'-H) 3.2 (m, 1, 2'-H), 4.75 (m, 3, 4'-H, and 5'-H), 5.62 (d, 1, 3'-H), 6.27 (t, 1, 1'-H, $J_{1',2'}=7$ Hz), 7.30–8.17 (m, 8, aromatic), 8.27 (d, 1, 6-H, $J_{6,4}=3$ Hz), 8.57 (d, 1, 4-H, $J_{4,6}=3$ Hz); IR (CHCl₃) Nu max 3000, 175, 1675, 1595, 1500, 1380, 1260, 1090, 1010 cm⁻¹; UV (CHCl₃) lambda max (epsilon max) 341 nm (3500). Anal. (C₂₃H₁₇Cl₂IN₂O₆) C, H, Cl, I, N.

0.41 g (0.67 mmol) of the beta anomer was added to a cold anhydrous solution of methanolic ammonia (25 ml); the flask was sealed and the mixture was stirred at 4° C. for 5 hours. The reaction was complete as indicated by tlc (10% MeOH/CH₂Cl₂). The solution was concentrated to a syrup which was subsequently treated with acetone and chilled (-20° C.) to give, after several days, the crystalline material 1-(2-deoxy-β-D-ribofuranosyl)-5-iodo-2-pyrimidinone. The product was collected by filtration, washed with acetone and dried, to provide 100 mg (45.1%) of the pale yellow material, mp 160°–170° C. (decomposes). ¹H NMR (DMSO-d₆) delta 2.35 (m, 2, 2'-H), 3.65 (m, 2, 5'-H), 3.90 (m, 1, 4'-H), 4.22 (m, 1, 3'-H), 5.22 (t, 2, OH), 6.0 (t, 1, 1'-H); $J_{1',2'}=6$ Hz), 8.62 (d, 1, 6-H, $J_{6,4}=3$ Hz), 8.75 (d, 1, 4-H). IR (KBr) Nu max 3390, 3140, (broad), 2925, 1640, 1600 (shoulder), 1500, 1390, 1290, 1250, 1100, 1070 cm⁻¹; UV (MeOH) lambda max (epsilon max) 335 nm (2830). Anal. (C₉H₁₁IN₂O₄) C, H, I, N.

EXAMPLE 4

The substituted pyrimidinone of Example 3, 1-(2-deoxy)-β-D-ribofuranosyl)-5-iodo-2-pyrimidinone (IPdR) was tested in vivo. Three groups of five Swiss Webster Balb/C mice weighing from 19 to 23 grams were implanted with HSV-2 (333 strain) with a virus load of 5×10^5 plaque forming units.

Drug administration began one day following virus implant. The IPdR was administered subcutaneously to a first group of mice and intraperitoneally to a second group of mice. The third group of mice which performed as controls were not given any IPdR. The dosage of IPdR was at the rate of 100 mg/kg twice daily for 2.5 days for a total of 5 doses. The results are provided in Table 1 below:

TABLE 1

| GROUP | DAYS—SURVIVAL |
|-----------------|--|
| Control | 5 out of 5 died (9.4 days average) |
| Subcutaneous | 1 died on day 11; 2 died on day 13 |
| IPdR | (12 days average) 2 long term survivors* |
| Intraperitoneal | 1 died on day 17 |
| IPdR | 4 long term survivors* |

* >30 days

EXAMPLE 5

In accordance with the present invention, 1-(2-Deoxy-β-D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone is prepared essentially in accordance with the procedure set forth in "Synthesis and Antiviral

Activity of 1-(2-Deoxy-β-D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone" by Schroeder and Bardos and Cheng, Volume 24, page 109, January 1981. HeLa cells were infected in 1640 RPMI medium with herpes simplex type 1 (HSV-1) and independently with herpes simplex type 2 (HSV-2) virus at a multiplicity of 5 to 10 plaque forming units per cell. The composition of 1640 RPMI medium is reported in "Biological Activity of 5 Ethyl, 5 Propyl, and 5 Vinyl 2'-Deoxyuridine" by Cheng et al published in Antimicrobial Agents and Chemotherapy, Volume 10, beginning at page 119 (1976). 1640 RPMI medium is commercially available from Gibco Company, Grand Island, N.Y. After 1 hour, virus absorption, the drugs were added. Resulting cultures were analyzed for virus titer at 24 hours post infection as described in the procedure set forth in "Biological Activity of 5-Ethyl, 5-Propyl and 5-Vinyl 2'-uridine" by Cheng et al. The results are set forth in Table 2. The numbers set forth in Table 2 show the number of plaque forming units in the control which contained no methylmercapto compound and the number of units at concentrations of 100, 200 and 400 micromoles of the methylmercapto compound. The results clearly indicate substantial decrease in the number of plaque forming units in the presence of 1-(2-Deoxy-β-D-ribofuranosyl)-5-(methylmercapto)-2-pyrimidinone.

TABLE 2

| compound conc., μM | plaque-forming units/mL | |
|--------------------|-------------------------|-------------------|
| | HSV-1(Kos) | HSV-2 (333) |
| 0 | 1.5×10^7 | 1.2×10^7 |
| 100 | 6.4×10^7 | 1.3×10^7 |
| 200 | 1.7×10^6 | 3.1×10^6 |
| 400 | 3.4×10^5 | 3.2×10^5 |

EXAMPLE 6

The methylmercapto composition, as described in Example 5, was tested for binding affinity with thymidine kinase from various sources. Viruses which induce the production of thymidine kinase, induce thymidine kinase specific to the virus. Tests of the binding affinity of the methylmercapto compound with thymidine kinase extracted from human cells showed little binding affinity; whereas, the binding affinity of the methylmercapto compound with thymidine kinase extracted from cells infected with herpes simplex 1 virus and with Varicella zoster virus infected cells, showed great binding affinity. It is believed that the compound of the invention, in order to become active in inhibiting the replication of the virus, must become phosphorylated. For such phosphorylation to occur, the thymidine kinase must first bind to the compound. Since binding with thymidine kinase produced by the virus is much more efficient and effective than binding with thymidine kinase from other sources, phosphorylation of the compound occurs more rapidly in the presence of active viruses producing thymidine kinase. The compound, activated by phosphorylation, then is able to interfere with replication of the virus.

EXAMPLE 7

1-(2-Deoxy-α-D-ribofuranosyl)-5-(methyl)-2-pyrimidinone, also known as 4-Deoxythymidine, was prepared by thionation of the 4-oxo group of diacetylated thymidine with phosphorus pentasulfide, followed by desulfuration of the 4-thiothymidine derivative by Raney nickel reduction. The method for preparation of

the above described methyl compound is essentially the same as described by Wightman et al. in Collection of Czechoslovakian Chemical Communications, Volume 38, beginning at page 1381 (1973).

The above described methyl compound was tested for viral inhibition substantially in accordance with the method of Example 5 except that the concentrations were 50 and 100 micromolar. The methyl compound showed a 95.5% inhibition for HSV-1 at 50 micromoles when compared with an untreated control and an 87.9% inhibition for HSV-2 when compared with an untreated control. By comparison, methylmercapto compounds of Example 5 at the same 50 micromolar concentration showed only a 41.1% inhibition for HSV-1 virus and a 57.2% inhibition for HSV-2 virus. At 100 micromolar concentration, a 99% inhibition was shown for the methyl compound for HSV-1 virus and a 98.3% inhibition was shown for HSV-2 virus. Again, by comparison, the methylmercapto compound of Example 5 only showed a 83.8% inhibition for HSV-1 and a 79.3% inhibition for HSV-2.

EXAMPLE 8

Part A—Antiviral Activity

The effects of 1-(2-Deoxy- β -D-ribofuranosyl)-5-iodo-2-pyrimidinone (IPdR) of Example 3 were compared with 1-(2-Deoxy- β -D-ribofuranosyl)-5-fluoro-2-pyrimidinone (FPdR) on HSV-2 (strain 333) in Hela cells. Twenty four well plates (sterile) were plated with 10^6 Vero cells/well the day before infecting. 250 PFU of HSV-2 were used in each well. The test compounds, IPdR and FPdR, were added at zero times of post infection and the cultures incubated at 37° C. for 48 hours. At the end of the incubation period the media was removed from Vero cells. Plates were strained with 0.8% crystal violet in 50% ethanol for 15 minutes. The plaque was counted. Table 3, below, provides the results.

TABLE 3

| ANTIVIRAL ACTIVITY | | |
|---|----------------------|------|
| Concentration (μ M) of Compound | Plaque Reduction (%) | |
| | IPdR | FPdR |
| 100 | 51.1 | 50.5 |

Part B—Cytotoxicity

To compare the toxicity of FPdR and IPdR human nasopharyngeal carcinoma KB cells obtained from the American Type Culture Collection were maintained as a monolayer in RPMI 1640 supplemented with 5% fetal bovine serum, 10 mM HEPES buffer (pH 7.4) and karamycin (100 μ g/ml). Cells were maintained at 37° C. in a humidified atmosphere of 5% CO₂ in air. Cell numbers

were determined with an electronic particle counter (Particle Data, Inc., Elmhurst, Ill.).

For determination of drug effect on cell proliferation, cells were harvested from rapidly proliferating cultures by treatment with pancreatin. Cell number was determined and plated in 24 well (6 mm well diameter) plates at a concentration of 5×10^3 cells/ml. Drug exposures were initiated by a complete change of medium after overnight incubation. Following a 72 hour drug exposure 50 ml. of thiazolyl blue was added and incubated for a further 4 hour period. The media from the plates was aspirated completely and 250 ml DMSO was added to each well. The plates were placed on a shaker for 5 minutes and the blue solution transferred to 96 well plates and read at 540 nm with a scanning multiwell spectrophotometer (Biotek Instruments, Inc. Burlington, Vt.). Table 4, below, provides the relevant test data.

TABLE 4

| CYTOTOXICITY | | | |
|--------------|--------------------------|----------|------------------|
| Compound | Concentration (μ m) | % Growth | ID ₅₀ |
| FPdR | 1.0/102 | | 25 μ m |
| | 10/84 | | |
| | 25/49 | | |
| | 50/21 | | |
| IPdR | 100/75 | | > 200 μ m |
| | 200/80 | | |

The foregoing comparative test data shows that IPdR and FPdR are comparable in antiviral activity. However, Part B demonstrates that FPdR has a toxicity against the host cells which is significantly greater than IPdR. Accordingly, this data provides evidence that notwithstanding the equivalent antiviral activity of FPdR and IPdR the high level of toxicity of the 5-fluoro homologue renders it ineffective as a therapeutic agent.

EXAMPLE 9

In order to examine the activity of IPdR administered by various routes, i.e., oral, intraperitoneal (IP) and subcutaneous (SC), 6 to 8 week old Swiss-Webster female mice (Hilltop Laboratories, Chatsworth, Calif. weighing approximately 20 g were infected intraperitoneally on day 0 with 5×10^5 PFU of HSV-2 (Strain 333). The drugs were administered once a day for five days or twice daily (12 hour intervals) for 2.5 days beginning 24 hours postinfection. Mean body weight determinations of drug treated and control animals were made during the treatment period as an indicator of sublethal toxicity. Deaths were recorded through day 45. Table 5, below, summarizes the results.

TABLE 5

| ANTIVIRAL ACTIVITY OF IPdR IN MICE | | | | | |
|------------------------------------|-------|----------------------------|------------------------|--|--------------------|
| Drug (mg/kg) | Route | Schedule ^a | Survivors/Total | Mean Survival Time ^b (days) | % ILS ^c |
| Control | IP | 1 \times 5 | 1/10 (10) ^d | 9.6 \pm 1.1 ^e | — |
| EPdR (100) | IP | 1 \times 5 | 1/5 (20) | 9.0 \pm 0.8 | 94 |
| IPdR (100) | IP | 1 \times 5 | 7/10 (70) | 12.0 \pm 4.4 | 129 |
| Control | SC | 1 \times 5 | 0/5 (0) | 9.4 \pm 1.1 | — |
| IPdR (100) | SC | 1 \times 5 | 2/5 (40) | 12.3 \pm 1.2 | 131 |
| Control | IP | 2 \times 2 $\frac{1}{2}$ | 0/10 (0) | 9.3 \pm 1.1 | — |
| IPdR (10) | IP | 2 \times 2 $\frac{1}{2}$ | 0/5 (0) | 12.8 \pm 3.6 | 133 |
| IPdR (40) | IP | 2 \times 2 $\frac{1}{2}$ | 2/5 (40) | 10.0 \pm 1.0 | 108 |
| IPdR (100) | IP | 2 \times 2 $\frac{1}{2}$ | 6/10 (60) | 12.5 \pm 1.3 | 135 |
| Control | oral | 2 \times 2 $\frac{1}{2}$ | 4/20 (20) | 10.6 \pm 2.1 | — |
| IPdR (10) | oral | 2 \times 2 $\frac{1}{2}$ | 0/5 (0) | 12.8 \pm 3.6 | 123 |
| IPdR (40) | oral | 2 \times 2 $\frac{1}{2}$ | 2/5 (40) | 10.0 \pm 1.0 | 94 |
| IPdR (100) | oral | 2 \times 2 $\frac{1}{2}$ | 20/20 (100) | > 45 | — |

TABLE 5-continued

| ANTIVIRAL ACTIVITY OF IPdR IN MICE | | | | | |
|------------------------------------|-------|-----------------------|-----------------|--|--------------------|
| Drug (mg/kg) | Route | Schedule ^a | Survivors/Total | Mean Survival Time ^b (days) | % ILS ^c |
| DHPG ^h (40) | oral | 2 × 2½ | 20/20 (100) | >45 | — |
| Control ^f | oral | 2 × 2½ | 0/5 (0) | 10.0 ± 1.2 | — |
| IPdR (100) | oral | 2 × 2½ | 4/5 (80) | 14.0 | 140 |
| DHPG (40) | oral | 2 × 2½ | 2/5 (40) | 20.0 ± 7.0 | 200 |
| Control | oral | 2 × 2½ ^g | 0/5 (0) | 9.8 ± 1.3 | — |
| IPdR (100) | oral | 2 × 2½ | 5/5 (100) | >45 | — |
| DHPG (40) | oral | 2 × 2½ | 5/5 (100) | >45 | — |

^adaily doses × days
^bof the mice that died
^cmean life span of treated mice greater than mean life span of untreated mice in percent, excluding 45 day survivors.
^dpercent survival
^estandard deviation
^finoculation dose at 1 × 10⁶ PFU
^g72 hours after implant
^hDihydroxy propoxymethyl guanine

Table 5 provides further evidence that IPdR is active against HSV-2 in vivo. Most importantly, the potent antiviral activity of IPdR can be maintained when given orally. However, regardless of the route of administration IPdR can significantly increase the life span of the host infected with HSV-2. Also, the same potent antiviral effect of IPdR can be seen whether the drug is administered to the animals at the onset of the HSV infection or well after initiation of HSV-2 infection.

EXAMPLE 10

IPdR and 5-bromo-2-pyrimidinone-2-deoxyribonucleoside (BrPdR) were tested for antiherpes activity according to procedures described by Cheng, Y.-C., et al. *Antimicrob. Agents Chemother* (1976) 10, 119. Table 6, below, summarizes the results.

TABLE 6

| Antiherpes Activities of (2-Deoxy-β-D-ribofuranosyl)-5-bromo- and -5-iodo-2-pyrimidinone | | | | |
|---|------------------------|-----------------------|-----------------|---------------|
| plaque-forming units as % of control | | | | |
| virus | BrPdR (5a) (100 μM) | IPdR (5b) (100 μM) | BVdU (30 μM) | ACG (5 μM) |
| HSV-1 (KOS) | 0.6 | 0.27 | 0.1 | 6.5 |
| HSV-1 (CL101) | | 1.6 | 0.4 | 4 |
| HSV-2 (333) | 4.5 | 2.8 | 1.7 | |
| HSV-2 (CEU-G) | 7.1 | 2.3 | | |
| ACG ¹ (S1) | | 22 | 3.7 | 100 |

TABLE 6-continued

| Antiherpes Activities of (2-Deoxy-β-D-ribofuranosyl)-5-bromo- and -5-iodo-2-pyrimidinone | | | | |
|---|------------------------|-----------------------|-----------------|---------------|
| plaque-forming units as % of control | | | | |
| virus | BrPdR (5a) (100 μM) | IPdR (5b) (100 μM) | BVdU (30 μM) | ACG (5 μM) |
| ACG ¹ (Tr7) | | 2.6 | 0.8 | 85 |
| BVdU ² | | 14 | 52 | 0.7 |
| PFA (phosphonoformic acid) | | 0.1 | | 100 |

¹acyclovir resistant strain of HSV-1
²(bromovinyl) deoxyuridine

Test results showed that both BrPdR and IPdR have significant antiherpes activities against various strains or HSV-1 and HSV-2, the latter (IPdR) showing the higher activity. IPdR also has been shown to have stronger binding to the virus-specific thymidine kinase. IPdR was, in addition, tested and found to exhibit somewhat reduced but still significant activity against acyclovir (ACG) and BVdU resistant strains of HSV-1 in which the resistance was due to modification of the thymidine kinase. The more highly ACG-resistant strain (S1) showed more cross resistance towards IPdR than the one with a lower level of ACG resistance (Tr7). But, it is of interest that IPdR was still effective against the virus strain resistant to acyclovir, the foremost currently used antiherpes agent. Against PFA-resistant strains (modification in the DNA polymerase), IPdR retained full activity.

We claim:

1. 1-(2-Deoxy-β-D-ribofuranosyl)-5-(iodo)-2-pyrimidinone.

* * * * *

55

60

65