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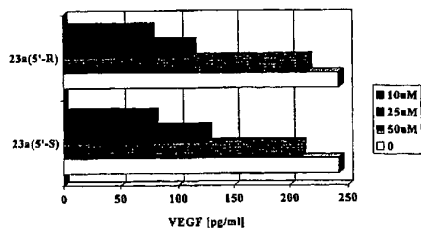


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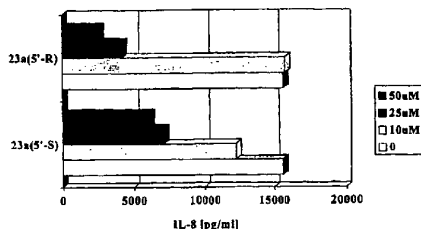
(54) Title: PYRROLO[2,3-d]PYRIMIDINE NUCLEOSIDE ANALOGS

Inhibition of VEGF Release in HTB 81 cells treated with 23a



(57) Abstract: Compositions and methods for pyrrolo[2,3-d]pyrimidine nucleoside analogs having substituents at the C4' and C5' positions of the ribofuranose moiety are presented. Contemplated compositions exhibit, among other things, anti-cancer and immunomodulating effects at reduced cytotoxicity.

Inhibition of IL-8 Release in HTB 81 cells treated with 23a



WO 01/27114 A1

PYRROLO[2,3-d]PYRIMIDINE NUCLEOSIDE ANALOGS**Field of The Invention**

The field of the invention is nucleoside analogs.

5 Background of The Invention

Nucleoside analogs have long been used as antimetabolites for treatment of cancers and viral infections. After entry into the cell, nucleoside analogs are frequently phosphorylated by nucleoside salvage pathways, in which the analogs are typically phosphorylated to the corresponding mono-, di-, and triphosphates. Among other intracellular destinations, triphosphorylated nucleoside analogs often are used as substrate for DNA or RNA polymerases and consequently incorporated into DNA or RNA. Where triphosphorylated nucleoside analogs are strong polymerase inhibitors, they may induce premature termination of a nascent nucleic acid molecule. Where triphosphorylated nucleoside analogs are incorporated into nucleic acid replicates or transcripts, gene expression or disruption of function may result.

15 On a more cellular level, the nucleoside analogs can also interfere with the cell cycle, and especially desirable effects of nucleoside analogs include induction of apoptosis of cancer cells. Furthermore, nucleoside analogs are also known to modulate certain immune responses.

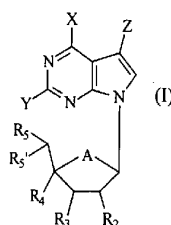
Various nucleoside analogs with relatively potent anti-cancer activity are known in the art. For example, known drugs include thymidylate synthase inhibitors such as 5-fluorouridine, adenosine deaminase inhibitors, including 2-chloroadenosine, and neplanocin A, which is an inhibitor of S-adenosylhomocysteine hydrolase. However, all or almost all of the known nucleoside analogs also imply a threat to normal mammalian cells, primarily because these nucleoside analogs lack adequate selectivity between normal cells and tumor cells. Unfortunately, lack of adequate selectivity is frequently associated with severe side effects, and therefore often limits the potential of such analog therapeutics.

25 Although there are various nucleoside analogs known in the art, all or almost all of them, suffer from one or more disadvantage. Therefore, there is still a need to provide improved methods and compositions for nucleoside analogs.

Summary of the Invention

The present invention is directed to nucleoside analogs with modifications on the sugar moieties of the pyrrolo[2,3-d]pyrimidine nucleoside analogs, which can significantly reduce the toxicity of the nucleoside analogs to the mammalian cells while they also provide significant cytotoxicity to cancer cells. These modifications include but are not limited to substitutions at the C4' and C5' positions of ribofuranose moieties. The present invention also demonstrates that certain pyrrolo[2,3-d]pyrimidine nucleoside analogs have desired immunomodulation effects, including enhancement of Type 1 cytokines such as IL-2 and suppression of Type 2 cytokines such as IL-4. These immunomodulation properties can be useful in anticancer, antiviral and autoimmune diseases, treating inflammation and preventing graft rejection.

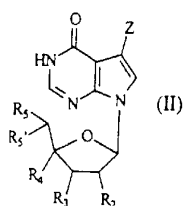
In one aspect of the inventive subject matter, the nucleoside analog is a pyrrolo[2,3]pyrimidine nucleoside having a structure according to the formula (I):



wherein A is O, S, or CH₂; X is H, NH₂ or OH; Y is H, halogen or NH₂; Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH₂, NHR, NR₂, CN, C(O)NH₂, COOH, COOR, CH₂NH₂, C(=NOH)NH₂, and C(=NH)NH₂, where R is alkyl, alkenyl, alkynyl, or aralkyl; R₂ and R₃ are independently selected from the group consisting of H, F, and OH; R₄ is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₄ optionally has at least one of a heteroatom and a functional group; R₅ is H, OH, OP(O)(OH)₂, P(O)(OH)₂, OP(O)(OR')₂, or P(O)(OR')₂, wherein R' is a masking group; and R₅ is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₅ has at least two carbon atoms, and optionally has at least one of a heteroatom and a functional group.

In another aspect of the inventive subject matter, the nucleoside analog is a pyrrolo[2,3d]pyrimidine nucleoside having a structure according to the formula (II):

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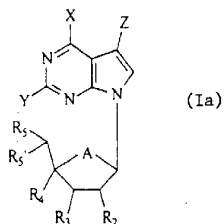


5 wherein Z is CN, C(O)NH₂, C(=NH)NH₂ or C(=NOH)NH₂ and R₄ and R₅ are independently selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₄ and R₅ independently and optionally contain at least one of a heteroatom and a functional group; with the proviso that R₄ and R₅ are not
10 together hydrogen.

In a further aspect of the inventive subject matter, contemplated compounds are utilized to inhibit tumor growth or to modulate Type 1 and Type 2 cytokine, and chemokine production.

15 In a further aspect of the invention there is provided a method of changing secretion of a cytokine from a cell, comprising:

providing a compound according to formula (Ia);



20 wherein A is O, S, or CH₂; X is H, NH₂ or OH; Y is H; halogen or NH₂;

Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH₂, NHR, NR₂, CN, C(O)NH₂, COOH, COOR, CH₂NH₂, C(=NOH)NH₂, and C(=NH)NH₂, where R is alkyl, alkenyl, alkynyl, or aralkyl;

25 R₂ and R₃ are independently selected from the group consisting of H, F, and OH;

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R_4 and R_5 are independently selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, and wherein R_4 and R_5 independently and optionally contain at least one of a heteroatom and a functional group;

5

R_5 is H, OH, $OP(O)(OH)_2$, $P(O)(OH)_2$, $OP(O)(OR')_2$, or $P(O)(OR')_2$, wherein R' is a masking group; and

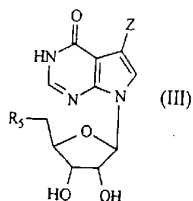
presenting the cell with the compound at a concentration effective to change the secretion of the cytokine.

10

In yet a further aspect of the invention there is provided a method of changing secretion of a cytokine from a cell, comprising:

providing a compound according to formula (III)

15



wherein Z is CN, $C(O)NH_2$, $C(=NH)NH_2$, $C(=NNH_2)NH_2$, or $-C(=NOH)NH_2$; wherein R_5 is H, OH, $OP(O)(OH)_2$, $P(O)(OH)_2$, $OP(O)(OR')_2$, or $P(O)(OR')_2$, wherein R' is a masking group; and

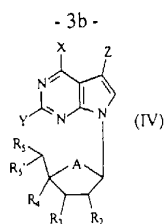
presenting the cell with the compound at a concentration effective to change the secretion of the cytokine.

25

In yet a further aspect of the invention there is provided a method of reducing growth of a hyperproliferative cell, comprising:

providing a compound according to formula (IV);

30



5 wherein A is O, S, or CH₂; X is H, NH₂ or OH; Y is H, halogen or NH₂;

Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH₂, NHR, NR₂, CN, C(O)NH₂, COOH, COOR, CH₂NH₂, C(=NOH)NH₂, and C(=NH)NH₂, where R is alkyl, alkenyl, alkynyl, or aralkyl;

10

R₂ and R₃ are independently selected from the group consisting of H, F, and OH;

R₄ is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₄ optionally contains at least one of a heteroatom and a functional

15 group;

R₅ is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₅ has at least two carbon atoms, and optionally contains at least one of a heteroatom and a functional group;

20

R₅ is H, OH, OP(O)(OH)₂, P(O)(OH)₂, OP(O)(OR')₂, or P(O)(OR')₂, wherein R' is a masking group, and presenting the hyperproliferative cell with the compound at a concentration effective to reduce the growth of the hyperproliferative cell.

25 In yet a further aspect of the invention there is provided a method of reducing a release of a growth factor from a cell, comprising;

 providing a compound of the invention; and

 presenting the cell with the compound at a concentration effective to reduce the release of the growth factor.

30

Various objects, features, aspects and advantages of the present invention will become

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more apparent from the following detailed description of preferred embodiments of the invention, along with accompanying drawings.

Throughout this specification and the claims which follow, unless the context requires
 5 otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an
 10 acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

BRIEF DESCRIPTION OF THE DRAWINGS

15 Fig. 1 is a first exemplary synthetic scheme of reactions included in the production of compounds according to the inventive subject matter.

Fig. 2 is a second exemplary synthetic scheme of reactions included in the production of
 20 compounds according to the inventive subject matter.

Fig. 3 is a third exemplary synthetic scheme of reactions included in the production of
 compounds according to the inventive subject matter.

Fig. 4 is a fourth exemplary synthetic scheme of reactions included in the production of
 25 compounds according to the inventive subject matter.

Fig. 5 is a fifth exemplary synthetic scheme of reactions included in the production of
 compounds according to the inventive subject matter.

Fig. 6 is a sixth exemplary synthetic scheme of reactions included in the production of compounds according to the inventive subject matter.

Fig. 7 depicts exemplary compounds according to the inventive subject matter.

Fig. 8A and 8B are graphs representing the effect of compounds according to the
5 inventive subject matter on the expression of Type 1 and Type 2 cytokines, respectively.

Fig. 9 is a table indicating cytotoxicity of various compounds according to the inventive subject matter.

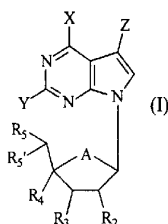
Fig. 10 is a table indicating rates of DNA synthesis in cells treated with of various compounds according to the inventive subject matter.

10 Fig. 11 is a graph depicting the inhibition of VEGF release from human prostate cancer cells upon treatment with compounds according to the inventive subject matter.

Fig. 12 is a graph depicting the inhibition of IL-8 release from human prostate cancer cells upon treatment with compounds according to the inventive subject matter.

15 **Detailed Description**

Pyrrolo[2,3-d]pyrimidine nucleoside analogs according to the general formulae (I) and (II) were found to have various biological effects on normal and hyperproliferative cells.



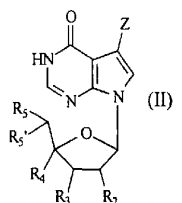
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wherein A is O, S, or CH₂; X is H, NH₂ or OH; Y is H, halogen or NH₂; Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH₂, NHR, NR₂, CN, C(O)NH₂, COOH, COOR, CH₂NH₂, C(=NOH)NH₂, and C(=NH)NH₂, where R is alkyl, alkenyl, alkynyl, or aralkyl; R₂ and R₃ are independently selected from the group consisting of H, F, and OH; R₄ is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₄ optionally has at least one of a heteroatom and a functional group; R₅ is H, OH, OP(O)(OH)₂, P(O)(OH)₂, OP(O)(OR')₂, or P(O)(OR')₂, wherein R' is a masking group; and R₅ is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₅ has at least two carbon atoms, and optionally has at least one of a heteroatom and a functional group.

It should be especially appreciated that the terms "alkyl", "alkenyl", "alkynyl", and "aralkyl" as used herein refer to both linear and branched species. With respect to the substituents R₂ and R₃, it should be appreciated that both R₂ and R₃ may be independently directed to the α- or β-face. Furthermore, where the substituents on C₅ are non-identical, substitution on C₅ may result in an R- or S-chiral center. The term "heteroatom", as used herein, refers to non-carbon atoms in an organic molecule, and particularly contemplated heteroatoms include halogens, nitrogen, oxygen, and sulfur. The term "functional group" as used herein refers to a reactive bond (e.g., double or triple bond) or reactive group (e.g., -OH, -SH, -NH₂, -N₃, -CN, COOH, -CHO, -CONH₂, etc.).

Particularly contemplated pyrrolo[2,3-d]pyrimidine nucleoside analogs are those according to formula (I) wherein Z is CN, C(O)NH₂, or C(=NH)NH₂, and wherein R₅ has at least two carbon atoms and is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl.

The pyrrolo[2,3-d]pyrimidine nucleoside analog according to formula (II) has the following structure:



5

AMENDED SHEET

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wherein Z is CN, C(O)NH₂, C(=NH)NH₂, or C(=NOH)NH₂ and R₄ and R₅ are independently selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₄ and R₅ independently and optionally contain at least one of a heteroatom and a functional group; with the proviso that R₄ and R₅ are not
 5 together hydrogen; and wherein the remaining substituents are as defined in formula (I).

It should be appreciated, however, that compounds according to the inventive subject matter may also be in forms and formulations other than previously described, and especially contemplated forms include prodrug forms or otherwise modified forms in
 10 which contemplated molecules are chemically and/or enzymatically modified to improve pharmacological and/or pharmacodynamical properties, including higher specificity towards target organs, cells or subcellular compartments and increased half-life time in the organism.

For example, cholesterol adducts may be formed to increase target specificity towards the liver, or apolipoprotein adducts may be formed to enhance penetration of the modified drug across the blood brain barrier to the brain. In another example, receptor ligand complexes may be synthesized to target the modified drug to a particular cell expressing a receptor specific for the ligand. Alternatively, antibody or antibody fragment
 20 complexes may be formed to increase selective delivery of the modified drug to a subcellular location. There are many prodrug and modified forms known in the art. Examples of prodrugs include charged or uncharged groups, lipophilic or polar groups may be added to contemplated molecules to increase the half-life time in serum or other target organs and/or cells. In further examples, it should be appreciated that the contemplated
 25 compounds, where phosphorylated at the C₅ atom, may also be di-, or tri-phosphorylated, or incorporate a thiophosphate.

While it is generally preferred that contemplated compounds have a sugar moiety in the D-configuration, it is also contemplated that the compounds may have a sugar
 30 moiety in the L-configuration. Further stereochemical aspects especially include R and S configurations at the C₅ atom where appropriate, and it should be appreciated that the substituents in contemplated compounds may be directed to α or β phase.

It should also be appreciated that contemplated compounds can be formulated in various formulations, including liquid, syrup or gel forms (e.g., for injection, ingestion, or topical administration) and solid forms (e.g., for ingestion, injection, or deposition in a body cavity). For example, where it is contemplated that compounds according to the inventive subject matter are

5 instable in the gastric environment, injection of a preferably isotonic solution is particularly contemplated. Alternatively, intranasal application or inhalation of a liquid form may be appropriate to circumvent acid degradation. On the other hand, where contemplated compounds are known to be resistant to digestive degradation, contemplated forms may be administered in form of a syrup or tablet. Depending on the particular use, contemplated compounds may also be

10 formulated for topical or transdermal applications. There are many more formulations known in the art, all of which are also contemplated suitable in conjunction with the inventive subject matter presented herein, and particularly contemplated formulations are described in "Drug Products for Clinical Trials: An Intl. Guide to Formulation, Production, Quality Control" by Donald C. Monkhouse and Christopher T. Rhodes (Editors); ISBN:082479852X.

15 It should still further be appreciated that contemplated compounds and formulations may include functional and non-functional additives. For example, where transcutaneous drug delivery is desired, skin penetration enhancers may be added. Alternatively, pharmaceuticals including cytostatic, antiviral, or immunomodulatory agents, may be added to synergistically or additively improve the function of contemplated compounds. Examples for non-functional

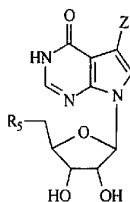
20 additives include fillers, antioxidants, flavor, or color agents to enhance a particular quality of contemplated compounds.

With respect to the concentration of contemplated compounds, it is preferred that the concentration is in a range of approximately 1 μ M to about 100 μ M when measured at the site of action. However, and particularly where the affinity of contemplated compounds is below 1 μ M,

25 appropriate concentrations may also be in the range of 999nM to 10nM, and less. On the other hand, where contemplated compounds exhibit relatively short half-life times, or have a high turnover, contemplated concentrations may be 0.1mM and 100mM, and more. Consequently, the dosage of contemplated compounds may vary significantly, but appropriate dosages can readily be determined in *in vitro* or animal experiments.

Among the various biological effects of both 5'- and 4'-modified pyrrolopyrimidine nucleoside analogs, particularly significant biological effects include modulation of the Type 1 and Type 2 cytokine production, control of neoplastic conditions (*i.e.*, reduction of DNA synthesis or reduction in cell growth), and reduction of chemokine and growth factor release as described below.

Consequently, a contemplated method of changing secretion of a cytokine from a cell may comprise a step in which a compound according to formula (I) is provided and has a further step in which the cell is presented with the compound according to formula (I) at a concentration effective to change the secretion of the cytokine. While all possible combinations of substituents in formula (I) are generally contemplated, particularly contemplated compounds are compounds according to formula (I) wherein R_4 and R_5 are independently selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, and wherein R_4 and R_5 independently and optionally contain at least one of a heteroatom and a functional group, with the remaining substituents as defined above in formula (I). In an alternative aspect, the compound employed to change the secretion of a cytokine from a cell may also be a compound according to the following structure:



wherein Z is CN, $C(O)NH_2$, $C(=NH)NH_2$, $C(=NNH_2)NH_2$, or $-C(=NOH)NH_2$, and wherein R_5 is H, OH, $OP(O)(OH)_2$, $P(O)(OH)_2$, $OP(O)(OR')_2$, or $P(O)(OR')_2$, with R' being a masking group.

Contemplated cytokines particularly include Type 1 (*e.g.*, $IFN\gamma$) and Type 2 (*e.g.*, IL-4) cytokines. With respect to the cells, it is contemplated that all cells known to produce and/or secrete cytokines are appropriate, however, especially contemplated cells include lymphocytes and cancer cells (*e.g.*, prostate cancer cells, *infra*).

In a further aspect of the inventive subject matter, a method of reducing growth of a hyperproliferative cell may comprise a step in which a compound according to formula (I) is provided, and another step in which the hyperproliferative cell is presented with the compound at a concentration effective to reduce the growth of the hyperproliferative cell. Particularly
5 preferred compounds include compounds according to formula (I) wherein R_4 is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R_4 optionally contains at least one of a heteroatom and a functional group, and wherein R_5 is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl,
10 wherein R_5 has at least two carbon atoms, and optionally contains at least one of a heteroatom and a functional group, with the proviso that R_4 and R_5 are not together hydrogen, and with the remaining substituents as defined above in formula (I).

Particularly contemplated hyperproliferative cells include cancer cells, and an especially contemplated cancer cell is a prostate cancer cell. While not wishing to be bound to a particular theory, it is contemplated that the reduction of growth comprises reduction of DNA synthesis.

15 In a still further aspect of the inventive subject matter, it is contemplated that a method of reducing a release of a growth factor from a cell has a step in which a compound according to formula (I) is provided, and another step in which the cell is presented with the compound at a concentration effective to reduce the release of the growth factor. It is contemplated that the release of various growth factors may be reduced by the method presented herein, however
20 reduction of VEGF release is especially contemplated. Similarly, while all cells known to secrete growth factors are contemplated in conjunction with the method presented herein, particularly contemplated cells include cancer cells, and especially prostate cancer cells.

With respect to the synthesis of contemplated compounds, it should be appreciated that pyrrolo[2,3-d]pyrimidine nucleoside analogs according to the inventive subject matter can be
25 synthesized via various synthetic routes, and the following procedures are provided by way of example only.

Synthesis of C5'-modified pyrrolo[2,3-d]pyrimidine nucleoside analogs

The 5'-substituted nucleoside analogs are prepared from the condensation of the pyrrolo[2,3-d]pyrimidine bases and the properly protected, 5'-substituted ribofuranoses. As shown in **Figure 1**, Compound 1, prepared according to a published procedure (Jones et al. Methods in carbohydrate Chemistry (edited by Whistler and Moffat), vol. VI, pp315-322, Academic Press, New York, (1972)), was treated with a variety of nucleophiles such as Grignard reagents to give compound 2, which was benzoylated and subsequently treated with trifluoroacetic acid to give compound 4. Benzoylation and the following treatment with acetic anhydride/acetic acid in the presence of sulfuric acid to give compound 6, which was used for condensation with pyrrolo[2,3-d]pyrimidine bases.

Compound 7 (Jones et al. Methods in carbohydrate Chemistry (edited by Whistler and Moffat), vol. VI, pp315-322, Academic Press, New York, (1972)), prepared according to a published procedure, was converted to a tosylate derivative, which was reduced with lithium aluminum hydride to give compound 8. By similar procedures shown in **Figure 1**, compound 8 was converted to compound 9. The condensation of 9 and the pyrrolo[2,3-d]pyrimidine 19 and the subsequent transformations as shown in scheme 2 gave compounds 10-15 as illustrated in **Figure 2**.

As shown in **Figure 3**, Compound 2 was converted to the sulfonate 16, which was subjected to nucleophilic replacement to give the configurationally inverted compound 17. Deprotection of the isopropylidene and the subsequent acetylation gave the tetraacetate 18. Condensation of the 5-C-substituted, protected ribofuranoses with nucleoside bases is depicted in **Figure 4**. 5-Cyanopyrrolo[2,3-d]pyrimidine 19, prepared according to a published procedure (Tolman et al. J. Org. Chem. 1969, 91, 2102-2108), was converted to the trimethylsilyl derivative and then condensed with compound 6 in the presence of trimethylsilyl triflate by a similar procedure described for toyocamycin (Sharma et al. Nucleosides Nucleotides 1993, 12, 643-648). The resulting coupling product was subjected to debromination through hydrogenation to give compound 20. Treatment of 20 with ammonia in anhydrous methanol gave compounds 21 and 23. Compound 21 was oxidized to give compound 22. Compound 23 was converted to the carboxamide derivative 24. Compound 23 and 24 were oxidized to give compound 25. Treatment of compound 25 with hydroxyamine yielded compound 26, which was hydrogenated

over Raney Nickel to give 27. Alternatively, compound 27 was also prepared by heating compound 25 with ammonia in a pressured bomb.

Synthesis of C4'-modified pyrrolo[2,3-d]pyrimidine nucleoside analogs

5 In Figure 5, compound 1 was treated with formaldehyde in aqueous sodium hydroxide to give 4'-hydroxymethyl derivative 28, which was selectively protected to afford compound 29. The subsequent protection with DMT and removal of TBS gave compound 31, which can be converted to a variety of substituents. The 4-C-substituted derivatives subjected to similar transformations as 5-C-substituted ribofuranoses (scheme 1) can be converted to compound 35,
10 which is used for condensation with nucleoside bases.

Similar to the C5'-substituted pyrrolopyrimidine nucleoside analogs, the 4'-substituted analogs 36 can be obtained by condensation of compound 35 with compound 19 as depicted in Figure 6. The subsequent transformations can give the 4'-C-substituted pyrrolopyrimidine nucleoside 37-42.

15 *Synthesis of 2'-modified, and other pyrrolo[2,3-d]pyrimidine nucleoside analogs*

The following pyrrolopyrimidine nucleoside analogs were prepared for biological testing, some of which were published (indicated as known compounds), and are shown in Figure 7. The known compounds 43, 44, 52-55, and 57 were prepared according to a published procedure (Hinshaw et al. J. Org. Chem. 1970, 92, 236-241). Compound 56 was prepared by hydrogenation
20 of compound 52. The known compound 49 (Krawczyk et al. Nucleosides Nucleotides 1989, 8, 97-115) was treated with sodium nitrite to give compound 50. The known compounds 45 and 48 were prepared according to a published procedure (Ramasamy et al. J. Heterocyclic Chem. 1988, 25, 1043-1046). Compound 45 was treated with ammonia-methanol to give compound 46 and hydrogenated to give compound 47. Compounds 58-63 were prepared from compound 45 by
25 similar procedures used for compounds 52-57. The known compound 64 (Krawczyk et al. Nucleosides Nucleotides 1989, 8, 97-115) was converted to compounds 65-67. The known compound 68 (Ramasamy et al. Tetrahedron 1986, 42, 5869-5878) was converted to compounds 69 and 70.

Preparation of human T-cells and activation in vitro

Peripheral blood mononuclear cells were isolated from healthy donors by density gradient centrifugation followed by T cell enrichment using Lymphokwik (One Lambda, Canoga Park CA). Contaminating monocytes were removed by adherence to plastic. Purified T cells were > 99% CD2+ , <1% HLA-DR+ and < 5% CD25+ and were maintained in RPMI-AP5 (RPMI1640 medium containing 5% autologous plasma, 1% L-glutamine, 1% penicillin/streptomycin and 0.05% 2-mercaptoethanol). For determination of cytokine protein levels, T-cells (0.2×10^6 cells in a volume of 0.2 ml) were activated by the addition of 2 ng phorbol myristate acetate plus 0.1 mg ionomycin (PMA-ION, both from Calbiochem, San Diego, CA) and incubated in 96 well plates in the presence of 0 or 10 μ M of various guanosine nucleosides for 48 h at 37°C. Following activation, supernatants were analyzed for cell-derived cytokine production.

Extracellular cytokine analyses.

Human cytokine levels were determined in cell supernatants, following appropriate dilution, using ELISA kits specific for IFN γ and IL-4 (Biosource, Camarillo, CA). All ELISA results were expressed as pg/ml.

Effect of pyrrolo-[2,3-d]pyrimidine nucleoside analogs on extracellular cytokine levels in activated human T cells.

The effect of pyrrolo-[2,3-d]pyrimidine nucleoside analogs at 0 and 10 μ M, on PMA/ionomycin stimulated T cell expression of the Type 1 cytokine, IFN γ , and the Type 2 cytokine, IL-4, is shown in **Figures 8A and 8B** for 5 individual human donors. Cytokine levels were determined in cell free supernatants by ELISA. The most potent effect was observed with 7-b-D-ribofuransyl-4-oxopyrrolo-[2,3-d] pyrimidine-5-carboxamide. This compound enhanced activated IL-4 production by $498\% \pm 83$ and suppressed IFN γ by $43\% \pm 4$ of the activated control levels of each cytokine. Data are shown as percentage of activated control calculated as the ratio of activated T cell cytokine level in the presence of test nucleosides over the cytokine level of untreated activated T cells $\times 100\%$. Zero effect on cytokine levels by test nucleosides would give a percentage of activated control value of 100 %. The absolute level (pg/ml $\pm$ standard deviation) of PMA-ION-induced cytokine secretion was for IFN γ , 22954 ± 3391 ; and for IL-4, 162 ± 40 . Resting levels were < 30 pg/ml for all cytokines tested.

Cytotoxicity of the pyrrolo[2,3-d]pyrimidine nucleoside analogs in vitro

The pyrrolo[2,3-d]pyrimidine nucleoside analogs of the present invention are bioactive since they indicate some level of cytotoxicity in vitro. In these studies, the compounds tested were applied to cell culture of normal human fibroblasts, human Prostate cancer cells 81, human Melanoma cancer cells 140, Human Lung Cancer cells 177, and human Ovarian Cancer cells R and NR (all available from ATCC). In these experiments cells were plated at density of 2000 cells per 200 μ l of medium per well (96-well plate). The compounds tested were applied to the wells once, at concentration range 0.78-100 μ M, just after plating of cells. The colorimetric cytotoxicity assay MTS was performed after 72 hrs of treatment. EC50 was calculated based on readings collected and they are presented in Figure 9. Several compounds indicate lack of cytotoxicity in concentration below 100 μ M. In such cases EC50 is marked as >100. In other cases, EC50 indicates the concentration of the compound tested needed to damage 50% of cell population.

The pyrrolo[2,3-d]pyrimidine nucleoside analogs inhibit DNA synthesis in cells cultured in vitro in a dose-dependent manner

Analogues of pyrrolo[2,3-d]pyrimidine nucleoside inhibit growth of human cells cultured *in vitro* as measured by the level of DNA. The experimental setup was the same as described above. The compounds were given once and DNA level was measured after 72 hrs. At that time, half of the medium was removed from culture wells and replaced by pure water. After that, the cells were transferred to -70°C for at least 12 hrs. In the next step, cells were transferred back from -70°C to room temperature and 1 μ M of Hoechst 33342 was given to each well. After 2 hrs of incubation, the fluorescence signal (360-530nm) was measured. According to this method, intensity of fluorescence is proportional to amount of DNA due to presence of DNA-Hoechst 33342 complex formed. The results are presented in Figure 10. The numbers express fold of DNA amount increase compared to amount of DNA at the beginning of experiment (2 hrs after plating of cells). In the untreated prostate cancer cells and normal cells the DNA level increased 5.78 and 4.47 times, respectively, during 72 hrs of culture.

The compounds 23a(5'-R) and 23a(5'-S) inhibit secretion of VEGF from Human Prostate Cancer in vitro.

The compounds 23a(5'-R) and 23a(5'-S) are potent in inhibition of secretion of Vascular Endothelial Growth Factor (VEGF) from Human Prostate cancer cells, HTB81. VEGF is
5 recognized as angiogenesis marker since this molecule is crucial for migration and growth of endothelial cells and microvessel formation in vivo. In order to prove this, 0.5×10^5 of the cells were plated in 5 ml of culture medium into a 10cm diameter petri dish. The compounds were applied just after plating for 72 hrs. After that, the medium was collected and the level of VEGF was measured using VEGF Elisa Assay (R&D Systems) and expressed as pg of VEGF per ml of
10 the medium. The results are presented in Figure 11. According to these results, both compounds inhibit secretion of VEGF in a dose-dependent manner.

The compounds 23a(5'-R) and 23a(5'-S) inhibit release of IL-8 from human prostate cancer cells cultured in vitro.

15 The compound 23a(5'-R) and 23a(5'-S) indicate an inhibitory effect on secretion of Interleukin-8 (IL-8) from human prostate cancer cells, HTB81. IL-8 belongs to the class of chemo-attractant chemokines (type alpha), which are involved in inflammation processes due to attraction of neutrophils. Chemokines, in general, are known to be produced by various types of cancer. It is proven in several studies that inhibition of chemokine production by cancer cells is
20 beneficial for the host. In order to prove the potency of these two compounds to inhibit IL-8 secretion from prostate cancer cells, prostate cancer cells HTB 81 were treated *in vitro* with compounds 23a in both the 5'-R and 5'-S configuration at concentrations indicated in the graph. The medium collected from the culture was analyzed for IL-8 level using IL-8 Elisa Assay from R&D Systems. According to the results collected, both compounds are able to inhibit secretion
25 of IL-8 in a dose-dependent manner, as depicted in Figure 12.

It should be appreciated, however, that the biological effects of contemplated compounds need not be limited to the particular effects as described above. In particular, it is contemplated that the compounds according to the inventive subject matter generally exhibit cytostatic effect in various hyperproliferative disorders, including localized and/or metastatic cancers (e.g.,
30 lymphomas and carcinomas), benign prostate hyperplasia, and keratoses. While the inventors found substantial biological effects on IL-4 (a Type 2 cytokine) and IFN γ (a Type 1 cytokine), it is generally contemplated that the compounds according to the inventive subject matter are biologically active in modulation of cytokines other than IL-4 and IFN- γ . It is especially

contemplated that the compounds may increase or decrease the expression/secretion of a particular cytokine or set of cytokines. Therefore, it is contemplated that compounds according to the inventive subject matter may modulate the immune system of an organism such that a more pronounced Type 1 or Type 2 response may be achieved. Consequently, it is contemplated
5 that the compounds according to the inventive subject matter may be effective to reduce the titer of a virus in a living system by either direct action as inhibitor of a viral polymerase and/or indirectly by activating the immune system to a particular humoral or cellular response. It is further contemplated that compounds according to the inventive subject matter may also be useful in reducing a response of an immune system towards an allo- or xenograft by reducing the
10 severity of the cellular response towards the allo- or xenograft.

Examples

The following protocols describe an exemplary synthesis of various compounds according to the inventive subject matter and are intended only to illustrate but not to limit the inventive concept presented herein.

15

Preparation of methyl 2,3-O-isopropylidene-5(R,S)-C-ethynyl-β-ribofuranoside (2b)

To a stirred solution of methyl 4-C,5-O-didehydro-2,3-O-isopropylidene-β-D-ribofuranoside (Jones et al. *Methods in Carbohydrate Chemistry* Vol 1, pp315-322 (1972), 4.00 g, 19.78 mmol) in anhydrous THF (20 mL) at -42 °C under argon was added dropwise
20 ethynylmagnesium bromide (0.5 M in THF, 80 mL, 40 mmol). Upon addition, the resulting mixture was slowly warmed up to 0 °C (~90 min.). The reaction was quenched by adding ice (50 g)/water (50 mL) and the mixture was stirred for 30 min. After neutralization with 10 % aq. acetic acid, the mixture was extracted with ethyl acetate twice. The combined organic layer was dried (Na₂SO₄) and concentrated. Chromatography on silica (ethyl acetate-hexanes 1:4) gave
25 3.48 g of the titled compound (R/S ratio 1:1) as a white solid. The following compounds were prepared in a similar fashion: Methyl 2,3-O-isopropylidene-5(R)-C-methyl-β-D-ribofuranoside (2a) was prepared from methyl 4-C,5-O-didehydro-2,3-O-isopropylidene-β-D-ribofuranoside and ethylmagnesium bromide. Methyl 2,3-O-isopropylidene-5(R)-C-vinyl-β-D-ribofuranoside (2c) was prepared from methyl 4-C,5-O-didehydro-2,3-O-isopropylidene-β-D-ribofuranoside
30 and vinylmagnesium bromide. Methyl 5(R)-C-allyl-2,3-O-isopropylidene-β-D-ribofuranoside (2d) was prepared from methyl 4-C,5-O-didehydro-2,3-O-isopropylidene-β-D-ribofuranoside and allylmagnesium bromide.

Preparation of methyl 2,3-O-isopropylidene-5-O-methanesulfonyl-5(R)-C-methyl-β-D-ribofuranoside (16)

To a stirred solution of methyl 2,3-O-isopropylidene-5(R)-C-methyl-β-D-ribofuranoside
5 (2a, 7.24 g, 33.17 mmol) in anhydrous pyridine (50 mL) at 0 °C was added methanesulfonyl
chloride (3.1 mL, 39.92 mmol). The resulting mixture was stirred at room temperature for 1 h,
cooled to 0 °C, quenched by adding water (1.0 mL), and stirred at room temperature for 30 min.
The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with brine
three times, dried (Na₂SO₄) and concentrated. Chromatography on silica (30% EtOAc in
10 hexanes) gave 8.62 g of the titled compound 16 as a colorless syrup.

Preparation of methyl 2,3-O-isopropylidene-5-O-acetyl-5(S)-C-methyl-β-D-ribofuranoside (17)

A stirred suspension of methyl 2,3-O-isopropylidene-5-O-methanesulfonyl-5(R)-C-
methyl-β-D-ribofuranoside (16, 8.62 g, 29.1 mmol) and NaOAc (anhydrous, 3.5 g, 42.5 mmol)
15 in anhydrous DMF (350 mL) was heated at 125 °C under argon for 4 days. The solvent was
evaporated and the residue chromatographed on silica (25% EtOAc in hexanes) to give 4.0 g of
the titled compound 17 as a white solid.

Preparation of methyl 2,3-O-isopropylidene-4-C-hydroxymethyl-β-D-ribofuranoside (28)

To a stirred solution of methyl 4-C,5-O-didehydro-2,3-O-isopropylidene-β-D-
ribofuranoside 1 (20.22 g, 100 mmol) in dioxane (380 mL) at 0 °C was added dropwise
formaldehyde (37 % solution, 76 mL) and then 2 M NaOH (188 mL). The resulting reaction
mixture was stirred at room temperature for 20 h, cooled to 0 °C, neutralized (10% acetic acid),
concentrated (~50 %), and extracted with methylene chloride twice. The combined organic layer
25 was dried (Na₂SO₄) and concentrated to dryness. Chromatography on silica (4 % methanol in
chloroform) gave 20.2 g of the titled compound 28 as a white solid.

Preparation of methyl 2,3-O-isopropylidene-5-deoxy-β-D-ribofuranoside (8)

To a stirred solution of methyl 2,3-O-isopropylidene-β-D-ribofuranoside (14.2 g, 70.0
30 mmol) in anhydrous pyridine (250 mL) at 10 °C was added in portions (over 30 min) p-
toluenesulfonyl chloride (19.1 g, 100 mmol). The resulting mixture was stirred at room
temperature for 18 h, cooled to 0 °C, quenched by adding water (5.0 mL), and stirred at room
temperature for 30 min. The solvent was evaporated. The residue was dissolved in ethyl acetate,

washed with brine three times, dried (Na_2SO_4) and concentrated to dryness. Chromatography on silica (ethyl acetate-hexanes 1:3) gave 24.1 g of the titled compound as a white solid.

To a stirred suspension of LiAlH_4 (4.58 g, 120.5 mmol) in anhydrous diethyl ether (120 mL) was added methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl- β -D-ribofuranoside (13.1 g, 36.55 mmol) in diethyl ether-toluene (2.5:1, 140 mL). The resulting mixture was refluxed for 22 h, cooled to room temperature, diluted with ethyl acetate (25 mL) quenched by adding water (5.0 mL). The solvent was evaporated. The residue was dissolved in ethyl acetate, washed with brine three times, dried (Na_2SO_4) and concentrated to dryness. Chromatography on silica (ethyl acetate hexanes 1:3) gave 3.58 g of the titled compound as a colorless liquid.

Preparation of methyl 5(R)-C-allyl-5-O-benzoyl-2,3-O-isopropylidene- β -D-ribofuranoside (3d)

To a stirred solution of methyl 5(R)-C-allyl-2,3-*O*-isopropylidene- β -D-ribofuranoside (4.49 g, 18.38 mmol) in anhydrous pyridine (40 mL) at 0 °C was added benzoyl chloride (2.7 mL, 23.0 mmol). The resulting mixture was stirred at room temperature for 18 h, cooled with ice, quenched by adding water (1 mL), and stirred at room temperature for 30 min. The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with brine three times, dried (Na_2SO_4) and concentrated. Chromatography on silica (12% ethyl acetate in hexanes) gave 6.26 g of the titled compound **3d** as a colorless syrup. The following compounds were prepared in a similar fashion: Methyl 5-*O*-benzoyl-5(R,S)-C-ethynyl-2,3-*O*-isopropylidene- β -D-ribofuranoside (**3b**, R/S ratio: 1:1) from methyl 5(R,S)-C-ethynyl-2,3-*O*-isopropylidene- β -D-ribofuranoside (**2b**). Methyl 4-*C*-benzoyloxymethyl-5-*O*-benzoyl-2,3-*O*-isopropylidene- β -D-ribofuranoside from methyl 2,3-*O*-isopropylidene-4-*C*-hydroxymethyl- β -D-ribofuranoside.

Preparation of methyl 5(R)-C-allyl-5-O-benzoyl- β -D-ribofuranoside (4d)

A solution of methyl 5(R)-C-allyl-5-*O*-benzoyl-2,3-*O*-isopropylidene- β -D-ribofuranoside (**3d**, 6.2 g, 17.8 mmol) in TFA- H_2O mixture (9:1) was stirred at 0 °C for 90 min and concentrated to dryness at 0°C. The residue was dissolved in methanol-toluene mixture (20 mL, 1:1) and concentrated to dryness. Chromatography on silica (ethyl acetate -hexanes 1:1) gave 3.70 g of the titled compound **4d** as a white solid. The following compounds were prepared in a similar fashion: Methyl 5-*O*-benzoyl-5(R,S)-C-ethynyl- β -D-ribofuranoside (**4b**, R/S ratio: 1:1) from methyl 5-*O*-benzoyl-5(R,S)-C-ethynyl-2,3-*O*-isopropylidene- β -D-ribofuranoside (**3b**).

Methyl 5-*O*-benzoyl-4-*C*-benzoyloxymethyl- β -D-ribofuranoside from methyl 5-*O*-benzoyl-4-*C*-benzoyloxymethyl-2,3-*O*-isopropylidene- β -D-ribofuranoside.

Preparation of methyl 5(R)-C-allyl-2,3,5-tri-O-benzoyl- β -D-ribofuranoside (5d)

5 To a stirred solution of methyl 5(R)-C-allyl-5-*O*-benzoyl- β -D-ribofuranoside (4d, 3.60 mg, 11.68 mmol) in anhydrous pyridine (80 mL) at 0 °C was added benzoyl chloride (3.0 mL, 25.84 mmol). The resulting mixture was stirred at room temperature for 18 h, cooled with ice, quenched by adding water (1 mL), then stirred at room temperature for 30 min. The mixture was concentrated, diluted with ethyl acetate, washed with brine three times, dried (Na₂SO₄) and
10 concentrated to dryness. Chromatography on silica (15% ethyl acetate in hexanes) gave 5.3 g of the titled compound 5d as a colorless syrup. The following compounds were prepared in a similar fashion: Methyl 5(R,S)-C-ethynyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranoside (5b, R/S ratio: 1:1) from methyl 5-*O*-benzoyl-5(R,S)-C-ethynyl- β -D-ribofuranoside (4b). Methyl 4-*C*-benzoyloxomethyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranoside from methyl 4-*C*-benzoyloxymethyl-5-*O*-
15 benzoyl- β -D-ribofuranoside.

Preparation of 1-O-methyl-2,3,5-tri-O-benzoyl-5(R)-C-vinyl- β -D-ribofuranose (5c)

A solution of methyl 2,3-*O*-isopropylidene-5(R)-C-vinyl- β -D-ribofuranoside (2c, 1.0 g, 4.3 mmol) in a mixture of trifluoroacetic acid and water (9:1, v/v, 11 mL) was stirred at 0 °C for
20 30 min and concentrated to dryness. The residue was dissolved in methanol and concentrated to dryness (3 times), then dissolved in pyridine and evaporated, and finally was dissolved in anhydrous pyridine (11 mL). To this solution was added benzoyl chloride (1.9 mL, 16 mmol). The reaction mixture was stirred at 25 °C for 16 h and poured into ice water (20 mL). The mixture was extracted with dichloromethane (20 mL) and the organic layer was dried over
25 sodium sulfate, and concentrated to dryness. The residue was chromatographed on silica (0-5% ethyl acetate in dichloromethane) to give 1.0 g of the titled compound 5c as a syrup.

30

Preparation of 1-O-acetyl-2,3,5-tri-O-benzoyl-5(R)-C-allyl-D-ribofuranose (6d)

To a stirred solution of methyl 5(R)-C-allyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranoside (5d, 4.0 g, 7.74 mmol) in acetic acid (14 mL) and acetic anhydride (1.75 mL, 18.36 mmol) at 0 °C

was added concentrated sulfuric acid (200 μ L, 3.79 mmol in 4.0 mL of acetic acid). The resulting mixture was stirred at room temperature for 20 h, cooled to 0 $^{\circ}$ C, diluted with cold ethyl acetate, washed with water, 5% aq. NaHCO_3 and then brine, dried (Na_2SO_4), and concentrated. Chromatography on silica (ethyl acetate-hexanes 1:4) gave 2.82 g of the titled compound 6d (α/β ratio: 1:2) as a colorless foam. The following compounds were prepared in a similar fashion: 1-*O*-Acetyl-5(R,S)-C-ethynyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose (6b, R/S ratio: 1:1 and α/β ratio: 1:2) from methyl 5(R,S)-C-ethynyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranoside (5b), 1-*O*-Acethyl-4-C-benzoyloxymethyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (α/β ratio: 1:3) from methyl 4-C-benzoyloxymethyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranoside, 5(R)-C-Methyl-1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose from methyl 2,3-*O*-isopropylidene-5(R)-C-methyl- β -D-ribofuranoside.

1,2,3,5-Tetra-*O*-acetyl-5(S)-C-methyl-D-ribofuranose 6a from methyl 5-*O*-acetyl-2,3-*O*-isopropylidene-5(R)-C-methyl- β -D-ribofuranoside, 5-deoxy-1,2,3-tri-*O*-acetyl-D-ribofuranose 9 from methyl 5-*O*-acetyl-2,3-*O*-isopropylidene- β -D-ribofuranoside, 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-5(R)-C-vinyl- β -D-ribofuranose 6e from methyl 2,3,5-tri-*O*-benzoyl-5(R)-C-vinyl- β -D-ribofuranoside.

*Preparation of 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-allyl- β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine*

A suspension of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (Tolman et al. J. Org. Chem. **1969**, 91, 2102-2108, 1.05 g, 4.41 mmol) and ammonium sulfate (50 mg) in HMDS (75 mL) and anhydrous *m*-xylene (25 mL) was refluxed under argon for 18 h. Solvents were evaporated and the residue was dried under vacuum. The residue was dissolved in anhydrous 1,2-dichloroethane (80 mL) and mixed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-5(R)-C-allyl-D-ribofuranose (2.00 g, 3.67 mmol). Under cooling with ice, TMSOTf (1.3 mL, 7.30 mmol in 5 mL of anhydrous 1,2-dichloroethane) was added. The mixture under argon was stirred at room temperature for 30 min, then refluxed for 90 h, quenched by pouring it (cold) onto ice/ NaHCO_3 (50 mL), and filtered. The organic layer was separated, dried (Na_2SO_4), and concentrated. Chromatography on silica (EtOAc-hexanes 2:3) gave 1.81 g of the titled compound as a colorless solid. The following compounds were prepared in a similar fashion: 4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R,S)-C-ethynyl- β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (R/S ratio: 1:1) was prepared from 1-*O*-acethyl-2,3,5-tri-*O*-benzoyl-5(R,S)-C-

ethynyl-D-ribofuranose and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine. 4-amino-6-bromo-5-cyano-7-(4-enzyloxomethyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was prepared from 1-*O*-acetyl-4-benzoyloxymethyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine. 4-Amino-6-bromo-5-cyano-7-(1,2,3,5-tetra-*O*-acetyl-5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was prepared from 1,2,3,5-tetra-*O*-acetyl-5(R)-C-methyl-D-ribofuranose and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine. 4-Amino-6-bromo-5-cyano-7-(1,2,3,5-tetra-*O*-acetyl-5(S)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was prepared from 1,2,3,5-tetra-*O*-acetyl-5(S)-C-methyl-D-ribofuranose and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine. 4-Amino-6-bromo-5-cyano-7-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was prepared from 1,2,3-tri-*O*-acetyl-5-deoxy-D-ribofuranose and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine. 4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-vinyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-5(R)-C-vinyl-β-D-ribofuranose and 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine.

*Preparation of 4-amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-allyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (20e)*

To a solution of 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-allyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (738 mg 1.0 mmol) in acetic acid (25 mL) was added zinc dust (1.04 g, 16.0 mmol) in two portions (one hour apart). The reaction mixture was stirred at room temperature for 20 h and filtered. The filtrate was evaporated to dryness and the residue chromatographed on silica (ethyl acetate-hexanes 1:1) to give 450 mg of titled compound **20e** as a colorless foam. The following compounds were prepared in a similar fashion: 4-Amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R,S)-C-ethynyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (R/S ratio: 1:1) **20b** from 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R,S)-C-ethynyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-vinyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **20c** from 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-vinyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine.

*Preparation of 4-amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-propyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (20f)*

A suspension of 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-allyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (400 mg, 0.54 mmol) and 10% Pd/C (100 mg, ~50% water) in dioxane (50 mL) and triethylamine (0.5 mL) was shaken in hydrogenation apparatus (H₂, 20 psi) for 4 h. The catalyst was filtered and washed (dioxane). The combined filtrate was concentrated and the residue chromatographed on silica (ethyl acetate-hexanes 1:1) to give 340 mg of the titled compound **20f** as a colorless foam. The following compounds were prepared in a similar fashion: 4-Amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R,S)-C-ethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (R/S ratio: 1:1) **20d** from 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R,S)-C-ethynyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(4-benzoyloxomethyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine from 4-amino-6-bromo-5-cyano-7-(4-benzoyloxomethyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(1,2,3,5-tetra-*O*-acetyl-5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **20a** from 4-Amino-6-bromo-5-cyano-7-(1,2,3,5-tetra-*O*-acetyl-5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(1,2,3,5-tetra-*O*-acetyl-5(S)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **20a** from 4-Amino-6-bromo-5-cyano-7-(1,2,3,5-tetra-*O*-acetyl-5(S)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(1,2,3-tri-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine from 4-amino-6-bromo-5-cyano-7-(1,2,3-tri-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(2,3-dideoxy-β-D-glicero-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine was prepared from 4-amino-5-cyano-7-(2,3-dideoxy-β-D-pent-2-enofuranosyl)pyrrolo[2,3-*d*]pyrimidine.

*Preparation of 4-amino-5-cyano-7-(5(R)-C-allyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (23e)*

A solution of 4-amino-5-cyano-7-(2,3,5-tri-*O*-benzoyl-5(R)-C-allyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (300 mg, 0.454 mmol) in methanol (40 mL) at 0 °C was saturated with ammonia. The solution stood at room temperature for 2 days. Solvent was evaporated and the residue together with NaOAc (anhydrous, 20 mg) was suspended in DMF (20 mL). The mixture was stirred under argon at 120 °C for 5 h. Solvent was evaporated. The residue was adsorbed onto silica gel and eluted from silica gel column (methanol-ethyl acetate 1:25) to give 145 mg of the titled compound as a colorless solid.

Before heating in DMF, the product contained two major compounds **21** and **23**, which could be separated by chromatography on silica gel. Compounds **21** were prepared through this procedure. The following compounds were prepared in a similar fashion: 4-Amino-5-cyano-7-(5(R)-C-propyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **23f** from 4-amino-5-cyano-7-(2,3,5-tri-O-benzoyl-5(R)-C-propyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(5(R,S)-C-ethynyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (R-S ratio: 1:1) **23b** from 4-amino-5-cyano-7-(2,3,5-tri-O-benzoyl-5(R,S)-C-ethynyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(5(R,S)-C-ethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (R-S ratio: 1:1) **23d** from 4-amino-5-cyano-7-(2,3,5-tri-O-benzoyl-5(R,S)-C-ethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(4-hydroxymethyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **33d** from 4-Amino-5-cyano-7-(4-benzoyloxomethyl-2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **23a(5'-R)** from 4-amino-5-cyano-7-(1,2,3,5-tetra-O-acetyl-5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(5(S)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **23a(5'-S)** from 4-amino-5-cyano-7-(1,2,3,5-tetra-O-acetyl-5(S)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **10** from 4-amino-5-cyano-7-(1,2,3-tri-O-acetyl-5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(5(R)-C-vinyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **23e** from 4-amino-5-cyano-7-(2,3,5-tri-O-benzoyl-5(R)-C-vinyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine.

*Preparation of 4-amino-5-cyano-7-(2,3-di-O-methanesulfonyl-5-O-tert-butylidiphenylsilyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine*

To a stirred solution of toyocamicin **43** (5.83 g, 20.0 mmol) in anhydrous pyridine (100 mL) at 0°C was added *tert*-butylchlorodiphenylsilane (6.2 mL, 24.0 mmol). The resulting mixture was stirred at room temperature for 18 h and then cooled to 0°C, and methanesulfonyl chloride (3.4 mL, 44.0 mmol) was added. The resulting mixture was stirred at room temperature for 2 h, cooled with ice, quenched by adding water (2 mL), and stirred at room temperature for 30 min. The solvent was evaporated. The residue was dissolved in ethyl acetate, washed with brine three times, dried (Na₂SO₄) and concentrated. Chromatography on silica (ethyl acetate/hexanes 3:2) gave 8.41 g of the titled compound as a colorless solid.

Preparation of 4-amino-5-cyano-7-(5-O-tert-butylidiphenylsilyl-2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine

Tellurium powder (200 mesh, 640 mg 5.0 mmol) under argon was sealed, mixed with lithium triethylborohydrate (1.0 M in THF, 11.25 mL, 11.25 mmol). The mixture was stirred at
 5 room temperature for 6 h and then cooled to 5 °C, and 4-amino-5-cyano-7-(2,3-di-O-methanesulfonyl-5-O-tert-butylidiphenylsilyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (1.40 g, 2.09 mmol) in THF (12 mL) was added. The resulting mixture was stirred at room temperature for 18 h, cooled with ice, quenched by adding water (0°C, 5 mL), and stirred at
 10 room temperature for 30 min. Solvent was evaporated and the residue extracted with ethyl acetate. The extracts were concentrated and the residue chromatographed on silica (15% ethyl acetate in hexanes) to give 640 mg of the titled compound as a colorless foam.

Preparation of 4-amino-5-cyano-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (49)

15 To a stirred solution of 4-amino-5-cyano-7-(5-O-tert-butylidiphenylsilyl-2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (2.55 g, 5.32 mmol) in anhydrous THF (100 mL) at 5 °C was added tetrabutylammonium fluoride (1.0 M in THF, 6.6 mL). The resulting mixture was stirred at room temperature for 3 h and concentrated. Chromatography on silica (6% methanol in ethyl acetate) gave 1.09 g of the titled compound 49 as a colorless solid.

20 *Preparation of 5-cyano-7-(5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-d]-4-pyrimidone (25a)*

To a stirred solution of 4-amino-5-cyano-7-(5(R)-C-methyl-β-D-ribofuranosyl)-pyrrolo-[2,3-d]pyrimidine (306 mg, 1.0 mmol) in water (30 mL) and acetic acid (2.0 mL) at 55 °C was added in portions sodium nitrite (590 mg, 8.55 mmol). The resulting mixture was stirred at 70 °
 25 C for 3 h and more sodium nitrite (300 mg, 4.30 mmol) was added. The mixture was stirred at same temperature for additional 18 h. Solvent was evaporated and the residue chromatographed on silica (12 % methanol in methylene chloride) to give 210 mg of the titled compound 25a(5'-R) as a colorless solid. Similarly, the following compounds were prepared: 4-Amino-5-cyano-7-(5(S)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-d]-4-pyrimidone 25a(5'-S) from
 30 4-amino-5-cyano-7-(5(S)-C-methyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine. 4-Amino-5-cyano-7-(β-D-arabinofuranosyl)pyrrolo[2,3-d]-4-pyrimidone 58 from 4-amino-5-cyano-7-(5-deoxy-β-D-arabinofuranosyl)pyrrolo[2,3-d]pyrimidine. 4-Amino-5-cyano-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-d]-4-pyrimidone 11 from 4-amino-5-cyano-7-(5-deoxy-β-D-

ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(2,3-dideoxy-2,3-didehydro-β-D-glycero-pento-furanosyl) pyrrolo[2,3-*d*]-4-pyrimidone **50** from 4-amino-5-cyano-7-(2,3-dideoxy-β-D-pent-2-enofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone **65** from 4-amino-5-cyano-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine. 4-Amino-5-cyano-7-(2-deoxy-β-D-furanosyl)pyrrolo[2,3-*d*]-4-pyrimidone **69** from 4-amino-5-cyano-7-(2-deoxy-β-D-eritropentofuranosyl)pyrrolo[2,3-*d*]pyrimidine.

10 *Preparation of 7-(5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (24a)*

A stirred suspension of 5-cyano-7-(5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (240 mg, 0.784 mmol), hydroxylamine hydrochloride (163 mg, 2.352 mmol), and potassium carbonate (162 mg, 1.176 mmol) in ethanol (50 mL) was refluxed under argon for 18 h. Precipitate was filtered and washed with warm ethanol. The filtrate was concentrated and the residue chromatographed on silica (20 % methanol in methylene chloride) to give 170 mg of the titled compound **26a(5'-R)** as a colorless solid. Similarly, the following compounds were prepared: 4-Amino-5-cyano-7-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime **60** from 4-amino-5-cyano-7-(5-deoxy-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone.

20 4-Amino-5-cyano-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime **13** from 4-amino-5-cyano-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone. 4-Amino-5-cyano-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime **51** from 4-amino-5-cyano-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone. 4-Amino-5-cyano-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime from 4-amino-5-cyano-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone.

25 *Preparation of 7-(5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine hydrochloride (27a)*

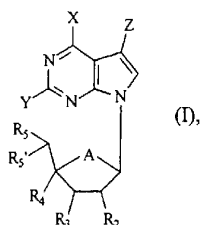
A suspension of 7-(5(R)-C-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (110 mg, 0.324 mmol), ammonium chloride (20 mg, 0.374 mmol), and Raney nickel (50% slurry in water, 200 mg) in water (75 mL) was shaken in a hydrogenation apparatus (H₂, 50 psi) at room temperature for 18 h. The catalyst was filtered and washed (warm water).

The combined filtrate was concentrated and the product was recrystallized from methanol to give 100 mg of the titled compound **27a(5'-R)** as a colorless solid. The following compounds were prepared in similar fashion: 4-Amino-5-cyano-7-(β -D-arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamide hydrochloride **63** from 4-amino-5-cyano-7-(5-deoxy- β -D-arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime. 4-Amino-5-cyano-7-(5-deoxy- β -D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamide hydrochloride **15** from 4-amino-5-cyano-7-(5-deoxy- β -D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime. 4-Amino-5-cyano-7-(2-deoxy- β -D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamide hydrochloride **70** from 4-amino-5-cyano-7-(2-deoxy- β -D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime.

Thus, specific embodiments and applications of pyrrolo[2,3-*d*]pyrimidine nucleoside analogs have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A nucleoside analog according to formula (I):



wherein A is O, S, or CH₂; X is H, NH₂ or OH; Y is H, halogen or NH₂;

Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH₂,

NHR, NR₂, CN, C(O)NH₂, COOH, COOR, CH₂NH₂, C(=NOH)NH₂, and

C(=NH)NH₂, where R is alkyl, alkenyl, alkynyl, or aralkyl;

R₂ and R₃ are independently selected from the group consisting of H, F, and OH;

R₄ is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₄ optionally has at least one of a heteroatom and a functional group;

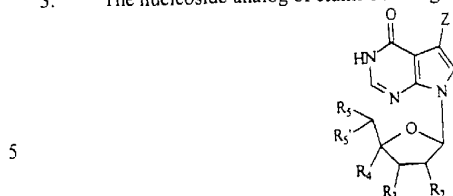
R₅ is H, OH, OP(O)(OH)₂, P(O)(OH)₂, OP(O)(OR')₂, or P(O)(OR')₂, wherein R' is a masking group; and

R₅ is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R₅ has at least two carbon atoms, and optionally has at least one of a heteroatom and a functional group.

2. The nucleoside analog of claim 1 wherein Z is CN, C(O)NH₂, or C(=NH)NH₂, and wherein R₅ has at least two carbon atoms and is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl.

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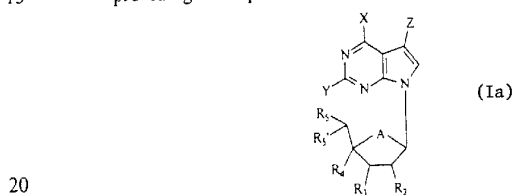
3. The nucleoside analog of claim 1 having the structure



wherein Z is CN, C(O)NH₂, C(=NH)NH₂, or C(=NOH)NH₂; and

- 10 R₄ is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and aralkyl, and R₅ is selected from the group consisting of an alkyl, an alkenyl, and alkynyl and an aralkyl, wherein R₄ and R₅ independently and optionally contain at least one of a heteroatom and a functional group.

4. A method of changing secretion of a cytokine from a cell, comprising;
15 providing a compound according to formula (Ia);



wherein A is O, S, or CH₂; X is H, NH₂ or OH; Y is H, halogen or NH₂;

- 25 Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH₂, NHR, NR₂, CN, C(O)NH₂, COOH, COOR, CH₂NH₂, C(=NOH)NH₂, and C(=NH)NH₂, where R is alkyl, alkenyl, alkynyl, or aralkyl;

R₂ and R₃ are independently selected from the group consisting of H, F, and OH;

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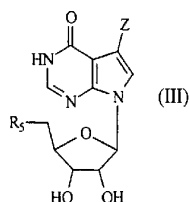
R_4 and R_5 are independently selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, and wherein R_4 and R_5 independently and optionally contain at least one of a heteroatom and a functional group;

R_5 is H, OH, $OP(O)(OH)_2$, $P(O)(OH)_2$, $OP(O)(OR')_2$, or $P(O)(OR')_2$, wherein R' is a masking group; and

presenting the cell with the compound at a concentration effective to change the secretion of the cytokine.

5. The method of claim 4 wherein the cytokine is a Type 1 cytokine.
6. The method of claim 5 wherein the Type 1 cytokine is $IFN\gamma$.
7. The method of claim 4 wherein the cytokine is a Type 2 cytokine.
8. The method of claim 7 wherein the Type 2 cytokine is IL-4.
9. The method of claim 4 wherein the cell is a lymphocyte.
10. The method of claim 4 wherein the cell is a cancer cell.
11. The method of claim 10 wherein the cancer cell is a prostate cancer cell.
12. A method of changing secretion of a cytokine from a cell, comprising:

providing a compound according to formula (III)



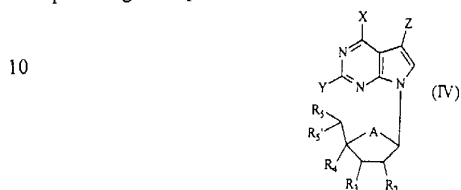
wherein Z is CN, $C(O)NH_2$, $C(=NH)NH_2$, $C(=NNH_2)NH_2$, or $-C(=NOH)NH_2$;

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wherein R_5 is H, OH, $OP(O)(OH)_2$, $P(O)(OH)_2$, $OP(O)(OR')_2$, or $P(O)(OR')_2$, wherein R' is a masking group; and

presenting the cell with the compound at a concentration effective to change the secretion
5 of the cytokine.

13. A method of reducing growth of a hyperproliferative cell, comprising:
providing a compound according to formula (IV);



wherein A is O, S, or CH_2 ; X is H, NH_2 or OH; Y is H, halogen or NH_2 ;

15 Z is selected from the group consisting of H, halogen, R, OH, OR, SH, SR, NH_2 , NHR, NR_2 , CN, $C(O)NH_2$, COOH, COOR, CH_2NH_2 , $C(=NOH)NH_2$, and $C(=NH)NH_2$, where R is alkyl, alkenyl, alkynyl, or aralkyl;

20 R_2 and R_3 are independently selected from the group consisting of H, F, and OH;

R_4 is selected from the group consisting of a hydrogen, an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R_4 optionally contains at least one of a heteroatom and a functional group;

25 R_5 is selected from the group consisting of an alkyl, an alkenyl, an alkynyl, and an aralkyl, wherein R_5 has at least two carbon atoms, and optionally contains at least one of a heteroatom and a functional group;

30 R_5 is H, OH, $OP(O)(OH)_2$, $P(O)(OH)_2$, $OP(O)(OR')_2$, or $P(O)(OR')_2$, wherein R' is a masking group, and presenting the hyperproliferative cell with the compound at a

- 30 -

concentration effective to reduce the growth of the hyperproliferative cell.

14. The method of claim 13 wherein the hyperproliferative cell is a cancer cell.

5 15. The method of claim 14 wherein the cancer cell is a prostate cancer cell.

16. The method of claim 13 wherein the reduction of growth comprises reduction of DNA synthesis.

10 17. A method of reducing a release of a growth factor from a cell, comprising;
providing a compound according to claim 1; and
presenting the cell with the compound at a concentration effective to reduce the release of
the growth factor.

15 18. The method of claim 17 wherein the growth factor is VEGF.

19. The method of claim 17 wherein the cell is a cancer cell.

20. The method of claim 19 wherein the cancer cell is a prostate cancer cell.

20 21. A nucleoside analog according to claim 1, substantially as hereinbefore described
with reference to the Examples.

22. A method of changing secretion of a cytokine from a cell according to claim 4 or
25 claim 12, substantially as hereinbefore described with reference to the Examples.

23. A method of reducing growth of a hyperproliferative cell according to claim 13,
substantially as hereinbefore described with reference to the Examples.

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24. A method of reducing release of a growth factor from a cell according to claim 17,
substantially as hereinbefore described with reference to the Examples.

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DATED this 24th day of November, 2003

ICN Pharmaceuticals, Inc.

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By DAVIES COLLISON CAVE
Patent Attorneys for the Applicants

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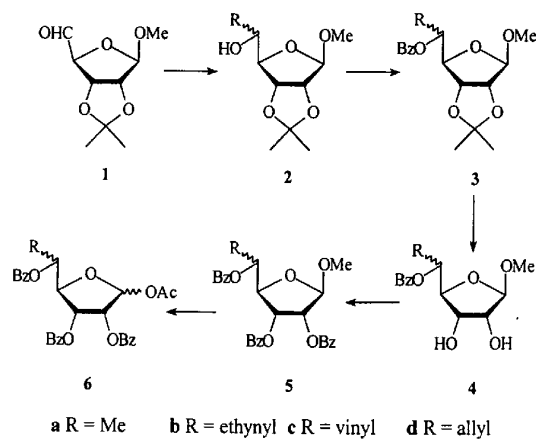


Figure 1

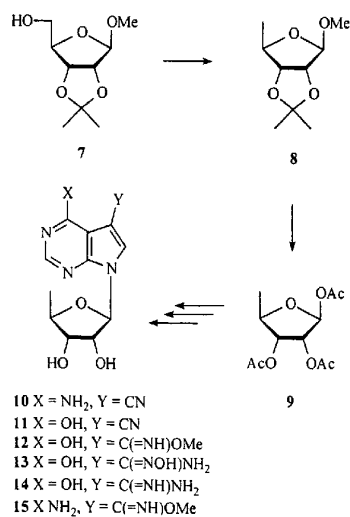


Figure 2

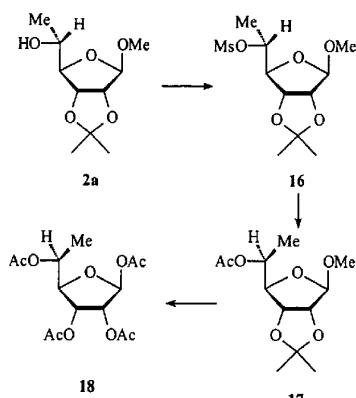
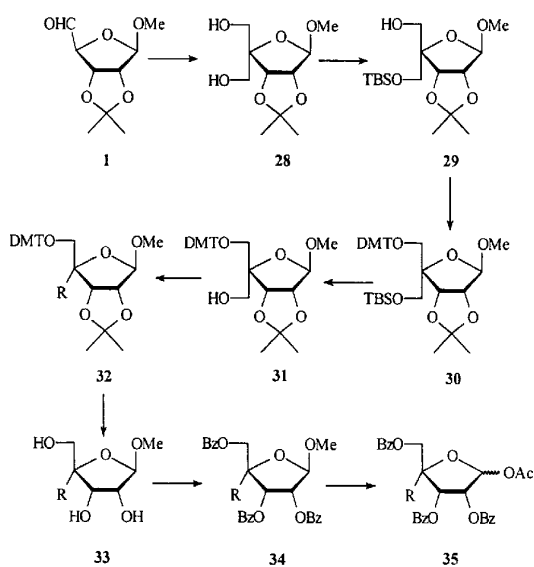


Figure 3



a R = methyl b R = vinyl
c R = ethyl d R = HOCH₂

Figure 5

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IPEA/US 26 MAR 2001

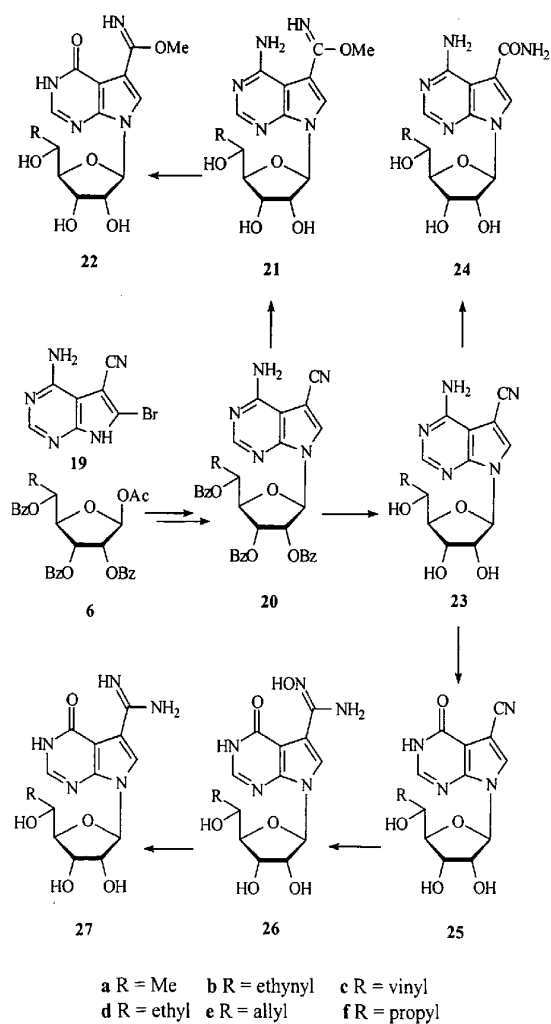


Figure 4

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AMENDED SHEET

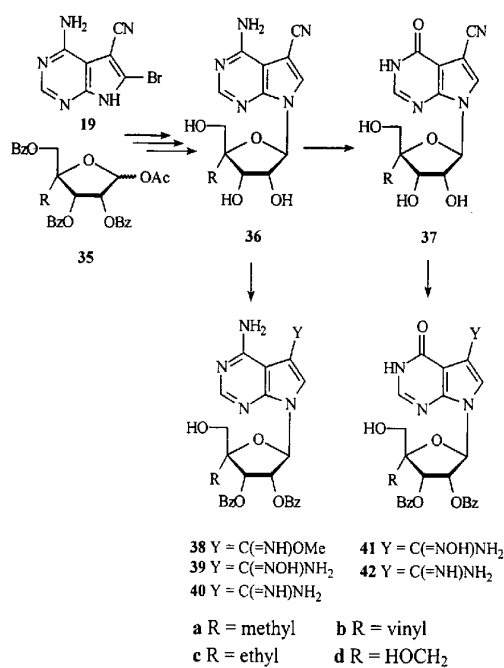
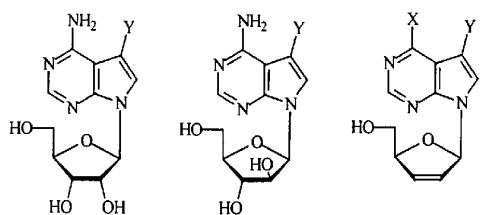
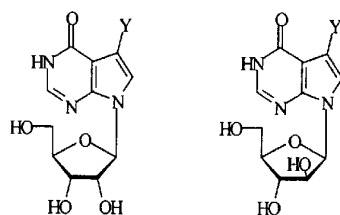


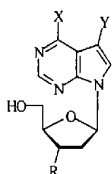
Figure 6



- 43 Y = CN
 44 Y = C(=NH)OMe
 45 Y = CN
 46 Y = C(=NH)OMe
 47 Y = CH₂NH₂
 48 Y = C(=NNH₂)NH₂
 49 X = NH₂, Y = CN
 50 X = OH, Y = CN
 51 X = OH
 Y = C(=NH)NH₂



- 52 Y = CN
 53 Y = C(=NH)OMe
 54 Y = C(=NOH)NH₂
 55 Y = C(=NH)NH₂
 56 Y = CH₂NH₂
 57 Y = C(=NNH₂)NH₂
 58 Y = CN
 59 Y = C(=NH)OMe
 60 Y = C(=NOH)NH₂
 61 Y = C(=NH)NH₂
 62 Y = CH₂NH₂
 63 Y = C(=NNH₂)NH₂



- 64 X = NH₂, Y = CN, R = H
 65 X = OH, Y = CN, R = H
 66 X = OH, Y = C(=NOH)NH₂, R = H
 67 X = OH, Y = C(=NH)NH₂, R = H
 68 X = NH₂, Y = CN, R = OH
 69 X = OH, Y = CN, R = OH
 70 X = OH, Y = C(=NH)NH₂, R = OH

Figure 7

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AMENDED SHEET

Figure 8A

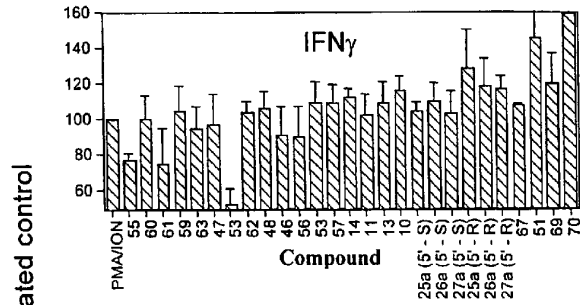


Figure 8B

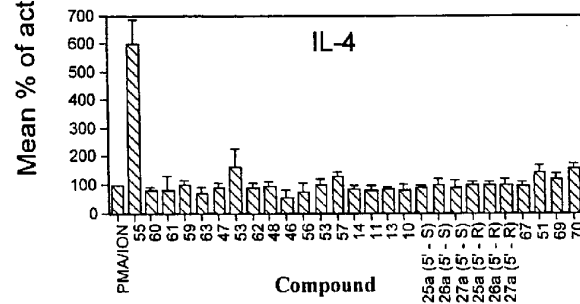


Table 1. Cytotoxicity of Analogs of the pyrrole[2,3-d]pyrimidine nucleoside - MTS assay						
Structure#	Prostate Cancer EC50[μM]	Normal Fibroblasts EC50[μM]	Melanoma Cancer EC50[μM]	Lung Cancer EC50	Ovarian R Ovarian NR EC50 EC50	
61:	12.5	50	40	34		
59:	>100	>100	>100	>100		
44:	0.07	0.08	0.06	0.02		
57:	0.01	0.08	0.01	0.05		
48:	>100	NT	>100	>100		
46:	60.1	NT	>100	99.3		
53:	98	39	82.5	97.5		
10:	>100	NT	>100	>100		
15:	>100	NT	>100	>100		
26a(5'-R)	>100	>100	>100	>100		
27a(5'-R)	>100	>100	>100	>100		
23a(5'-S)	44.2	45.6	51.8	30.1		
21a(5'-S)	98.2	>100	>100	90.9		
23a(5'-R)	20.4	45.5	43.9	24.8	12.1	43.4
21a(5'-R)	>100	>100	NT	NT		
24a(5'-S)	>100	39.6	87.3	80.7		
24a(5'-R)	70.1	34.9	70.1	30.8		
49:	5.6	3.3	NT	NT	5.7	5.1
64:	4.3	2.3	NT	NT	4.4	3.7
50:	>100	>100	>100	>100		
51:	>100	>100				
23e(5'-R)	50.6	>100				
23(5'-R)	45.9	>100				

Figure 9

Table 2. The inhibition of total DNA synthesis in cells of cells treated with analogs of pyrrole[2,3-d]pyrimidine nucleoside for 72 hrs at concentrations is dose-dependent												
Prostate Cancer Cells, HBL100												
Conc.	24a(5'-S)	24a(5'-R)	27a(5'-R)	24a(5'-R)	26a(5'-R)	27a(5'-R)	23a(5'-S)	21a(5'-S)	23a(5'-R)	21a(5'-R)		
1000μM	3.28	4.78	3.11	5.42	5.82	7.21	0.84	0.81	0.74	1.32		
50	3.41	3.34	4.09	5.04	5.31	5.88	1.2	2.84	0.89	3.18		
25	3.80	3.51	3.84	5.11	5.31	5.29	2.1	4.41	1.15	3.10		
12.5	3.69	3.69	3.69	5.25	5.25	5.37	2.9	4.31	3.23	3.81		
6.25	3.62	3.62	3.62	5.31	5.31	5.31	3.18	4.31	3.3	4.2		
3.125	3.89	3.89	3.89	5.31	5.31	5.31	4.8	4.31	3.8	4.97		
0.781	3.81	3.81	3.81	5.31	5.31	5.31	5.31	4.82	5.31			
0	5.78											
Normal Cells, Human Fibroblasts												
1000	3.5	4.35	3.47	4.18	4.31	2.33	0.83	1.65	1.29	3.75		
500	3.7	4.99	4	4.78	4.35	4.1	1.33	4.31	1.89	4.11		
250	4.32	4.63	4.32	4.11	4.6	4.3	4.5	4.51	5.7	4.71		
125	4.28	4.53	4.2	4.22	4.9	5.11	5.1	4.6	4.6	4.4		
62.5	4.18	4.35	4.18	5	5	5	5	4.6	4.6	4.51		
31.25	4.34	4.34	4.34	5.2	5.2	5.2	5.2	4.61	4.71	4.5		
0.781	4.29	4.38	4.29	5.2	5.2	5.2	5.2	4.61	4.71	4.71		
0	4.47											

Figure 10

Inhibition of VEGF Release in HTB 81 cells treated with 23a

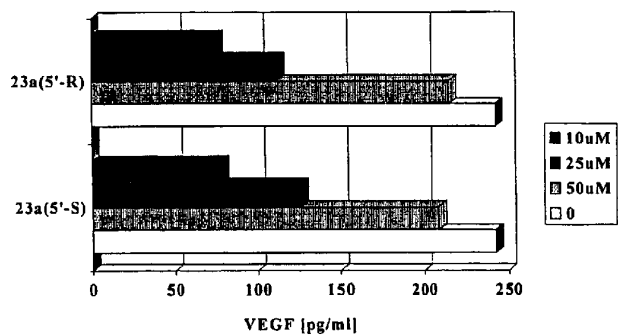


Figure 11

Inhibition of IL-8 Release in HTB 81 cells treated with 23a

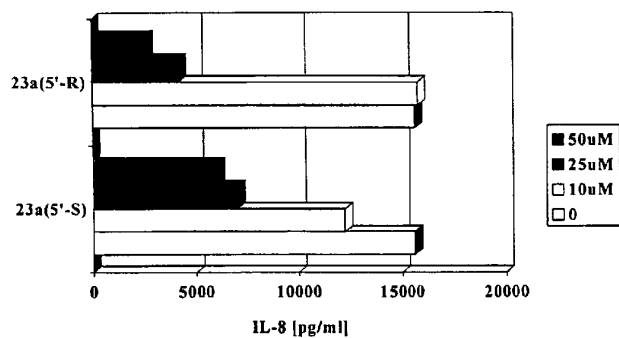


Figure 12