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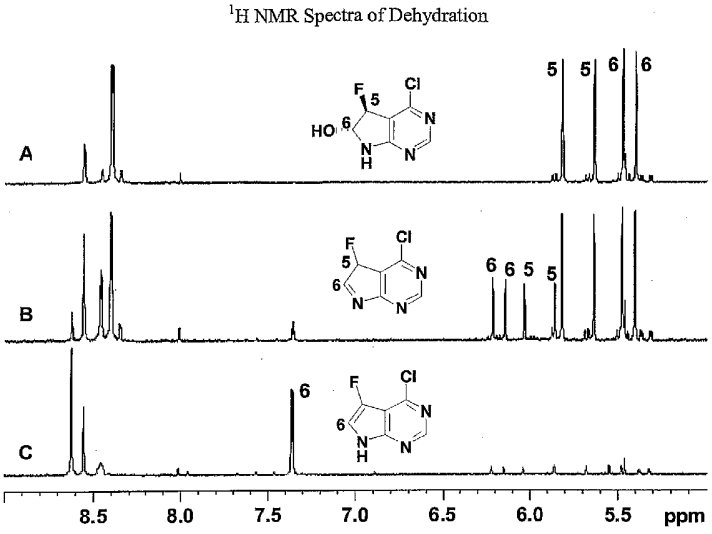

A) 25°C, 5 min; B) 70°C, 2.5h; C) 70°C, 14h.

(57) Abstract: 5-fluoro-pyrrolo[2,3-*d*]pyrimidine and 5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside compounds and methods for their preparation are disclosed.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHODS FOR THE PREPARATION OF 5-FLUORO-PYRROLO[2,3-*d*]PYRIMIDINE COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATION

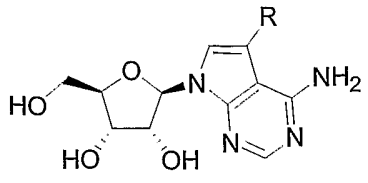
[0001] This application claims priority to U.S. Application No. 60/494,988, filed August 13, 2003, the disclosure of which is incorporated by reference in its entirety.

FIELD

[0002] This invention relates to 5-fluoropyrrolo[2,3-*d*]pyrimidine compounds and methods for their preparation.

BACKGROUND

[0003] Pyrrolo[2,3-*d*]pyrimidine nucleosides have received considerable interest as biologically active molecules (Townsend, L. B.; Editor *Chemistry of nucleosides and nucleotides, Vol. 2*, 1991; 468 pp., and *Vol. 3*, 1994; 553 pp.). In particular, extensive synthetic and biological studies have been carried out on the naturally occurring tubercidin and its 5-substituted derivatives, toyocamycin and sangivamycin. (Bergstrom, et al., *Journal of Medicinal Chemistry,* **27**: 285-292, 1984).



Tubercidin; R = H	5-Iodotubercidin; R = I
Toyocamycin; R = CN	5-Chlorotubercidin; R = Cl
Sangivamycin; R = $CONH_2$	5-Bromotubercidin; R = Br

[0004] Tubercidin is closely related in structure to adenosine and is rapidly converted to its 5'-monophosphate by adenosine kinase and subsequently to the higher phosphates (Miller, J. P. et al. *Biochemical and Biophysical Research Communications,* **55:** 843-849, 1973). A number of biological activities have been reported for tubercidin and its derivatives which include; *in vitro* cytotoxicity in mammalian cell strains, significant *in vivo* antitumor activity, inhibition of RNA and DNA virus replication and the inhibition of the growth of a variety of microorganisms (Anderson, J. D. et al. *Nucleosides & Nucleotides,* **8:** 1201-1216, 1989; Cottam, H. B. et al. *Journal of Medicinal Chemistry*, **28**: 1461-1467, 1985; Nichol, C. A.; et al. *Cancer Chemother., Proc. Takeda Int. Conf., Osaka* 185-195, 1967; De Clercq, E.; et al. *Antimicrobial Agents and Chemotherapy,* **30**: 719-724, 1986; De Clercq, E. et al. *Antimicrobial Agents and Chemotherapy,* **29:** 482-487, 1986; Pudlo, J. S. et al. *Journal of Medicinal Chemistry,* **31:** 2086-2092, 1988. Nichol, C. A. *Handbuch der Experimentellen Pharmakologie,* **38**: 434-457, 1975; U.S. Patent Nos. 6,342,501; 4,892,865).

[0005] Despite the reported antitumor activity of tubercidin, its toxicity has precluded its use in the clinic. Kazimierczuk, Z. et al. *Nucleic Acids Research,* **12**: 1179-1192, 1984. As a result, considerable effort has been expended in the search for derivatives with a greater selectivity in their biological response. For example, the related halogenated analogs such as 5-iodotubercidin, 5-bromotubercidin and 5-chlorotubercidin have been prepared, and have been found to have cytotoxic effects and antiviral activities (Kazimierczuk, Z. et al. *Nucleic Acids Research,* **12**: 1179-1192, 1984. Ugarkar, B. G. et al. *Journal of Medicinal Chemistry,* **43**: 2883-2893, 2000. Cook, A. F.; et al. *Nucleosides & Nucleotides,* **3**: 401-411, 1984. Hinshaw, B. C. et al., *Journal of Heterocyclic Chemistry,* **6**: 215-221, 1969. Nassiri, M. R. et al. *Antiviral Research,* **16**: 135-150, 1991). Exploration of the complete tubercidin pharmacophore necessitates the making and testing of the 5-fluoro analog. To our knowledge, however, the 5-fluoro analog of tubercidin has yet to be reported in the literature, perhaps due to the synthetic difficulties associated with direct electrophilic fluorinations.

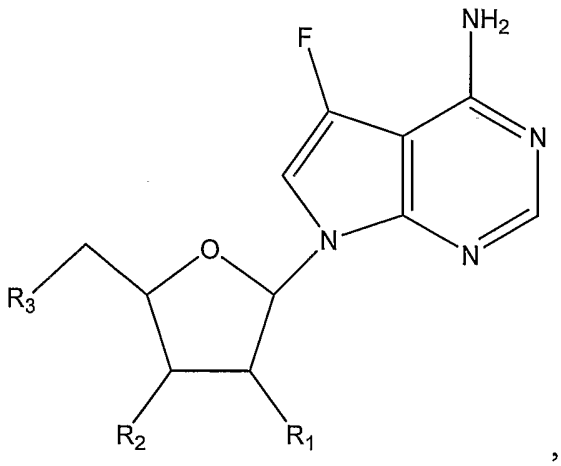
[0006] A need therefore exists for the methods of synthesis of such 5-fluoro analogs of tubercidin.

SUMMARY

[0007] The present invention is generally related to a method of synthesis of 5-fluoro-pyrrolo[2,3-*d*]pyrimidine and 5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside compounds. The method of synthesis relates to electrophilic fluorination of a 4-substituted pyrrolo[2,3-*d*]pyrimidine to yield 4-substituted-5-fluoro-pyrrolo[2,3-*d*]pyrimidine. Suitable 4-substituted pyrrolo[2,3-d]pyrimidines include 4-halo, 4-protected amino, 4-oxo and 4-alkyl where alkyl includes C1 to C6 alkyl. The invention more particularly relates to electrophilic fluorination of a 4-halogenated pyrrolo[2,3-*d*]pyrimidine to yield 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine. The invention more particularly relates to electrophilic fluorination of a 4-chloro-pyrrolo[2,3-*d*]pyrimidine to yield 4-chlopo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine. The present invention further relates to a method of synthesis by electrophilic fluorination of a 4-substituted pyrrolo[2,3-*d*]pyrimidine including 4-halogenated pyrrolo[2,3-*d*]pyrimidine particularly 4-chloro-pyrrolo[2,3-*d*]pyrimidine, culminating in the synthesis of 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside, *e.g.*, 5-fluorotubercidin.

[0008] The methods of the invention further provide compounds useful as intermediates in the systhesis of 5-fluorotubercidin. In one embodiment, the compound 5-fluoro-pyrrolo[2,3-*d*]pyrimidine is provided. In a further embodiment the compound 4-chloro-5-fluoro-7*H*-pyrrolo[2,3-d]pyrimidine is provided. In a further embodiment the compound 4-chloro-5-fluoro-5*H*-pyrrolo[2.3-d]pyrimidine is provided. In a further embodiment the compound 4-chloro-5-fluoro-6,7-dihydro-5*H*-pyrrolo[2,3-d]pyrimidin-6-ol is provided. In a further embodiment the compound 4-chloro-5-fluoro-7-(β-D-ribofuranosyl)pyrrolo[2.3-d]pyrimidine is provided.

[0009] The methods of the invention further provide nucleoside compounds of formula:



,

or a stereoisomer, prodrug, pharmaceutically acceptable salt, hydrate, solvate, acid salt hydrate, N-oxide or isomorphic crystalline form thereof, wherein R_1, R_2, and R_3 are selected, independently, from hydroxyl or a hydroxyl protecting group. These compounds can be used in

pharmaceutical compositions that comprises at least one pharmaceutically acceptable carrier or excipient and an effective amount of the nucleoside compound, wherein R is hydroxyl.

[0010] In a particular embodiment of the invention, the methods comprise the steps of providing 4-halo-pyrrolo[2,3-*d*]pyrimidine, and reacting the 4-halo-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine. In a further embodiment, methods comprise the steps of providing 4-chloro-pyrrolo[2,3-*d*]pyrimidine, and reacting the 4-chloro-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine. In a further detailed embodiment, the 4-chloro-pyrrolo[2,3-*d*]pyrimidine is reacted with the electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid. In a detailed embodiment, the electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate). In a further detailed embodiment, the reaction of the 4-chloro-pyrrolo[2,3-*d*]pyrimidine with the electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C. In a further detailed embodiment, the reaction with the electrophilic fluorinating reagent occurs for a time period of approximately 2 to approximately 14 hours.

[0011] In another embodiment, methods comprise the steps of providing 4-halo-pyrrolo[2,3-*d*]pyrimidine, reacting the 4-halo-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine, reacting the 4-halo-5-fluoro-pyrrolo [2,3-*d*]pyrimidine with a sugar derivative to form 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside, and reacting the 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside with ammonia to form 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside. In a detailed embodiment, the 4-halo-pyrrolo[2,3-*d*]pyrimidine is 4-chloropyrrolo[2,3-*d*]pyrimidine. In a further detailed embodiment, the 4-chloro-pyrrolo[2,3-*d*]pyrimidine is reacted with the electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid. In a further detailed embodiment, the electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate). In a further detailed embodiment, the reaction of the 4-chloro-pyrrolo[2,3-*d*]pyrimidine with the electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C. In a further detailed embodiment, the reaction with the electrophilic fluorinating reagent occurs for a time period of approximately 2 to approximately 14 hours. Sugar derivatives include β-*D*, β-*L*, α-*D* and α-*L* forms of ribo, xylo, lyxo and arabino sugars and further include 2', 3', 4' and 5' substituted derivatives of these sugars. In a detailed embodiment such sugar derivatives include 2' and 3' halo, alkoxyl and alkyl derivatives of these sugars. In a further detailed embodiment, the sugar derivative is a D-

ribofuranose derivative. For preparing 5-fluorotubercidin useful as the sugar derivative is 1-*O*-acetyl-2,3,5,-tri-*O*-benzoyl-β-D-ribofuranose.

[0012] In a further embodiment, methods comprise the steps of providing 4-chloro-pyrrolo[2,3-*d*]pyrimidine, reacting the 4-chloro-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine, reacting the 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine with an appropriate protected sugar derivative, *e.g.*, D-ribofuranose, to form 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside, and reacting the 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside with ammonia to form 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1. ^{1}H NMR Spectra of Dehydration.

DETAILED DESCRIPTION

[0014] In one aspect, the present invention relates to electrophilic fluorination of 4-substituted pyrrolo[2,3-*d*]pyrimidines particularly 4-halo-pyrrolo[2,3-*d*]pyrimidines and even more particularly 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidines. These compounds are useful intermediates for the synthesis of 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleosides, *e.g.*, 5-fluorotubercidin. Using the methods of the invention, 5-fluorotubercidin was prepared and evaluated in a variety of cell lines for antiproliferative activity.

[0015] Utilizing a method of the invention the conversion of 4-chloro-pyrrolo[2,3-d]pyrimidine to 4-chloro-5-fluoro-pyrrolo[2,3-d]pyrimidine was effected as a one pot synthesis. The electrophilic fluorination of 4-chloro-pyrrolo[2,3-*d*]pyrimidine (**1**) was studied culminating a 59% conversion of compound **1** to 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine (**2**) using the electrophilic fluorinating reagent, 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor, Aldrich Chemical Co.). This transformation proceeded via the 4-chloro-5,6-dihydro-5-fluoro-6-hydroxypyrrolo[2,3-*d*]pyrimidine (**3**) in a 9:1 trans:cis ratio. The trans isomer of compound **3** was studied by ^{1}H NMR and ^{19}F NMR, and the 5-H tautomer (**4**) was also identified. A modified Vorbruggen procedure using compound **2** and tetra-O-acetylribose gave 4-chloro-5-fluoro-7-(2,3,5,-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**6**) in a 65% yield. Treatment of compound **6** with ammonia in dioxane gave 5-fluorotubercidin (**7**). Other nucleosides are prepared in a like manner using compound **2** and an appropriate protected sugar. Such other nucleosides in addition to the above described β-*D*-ribo

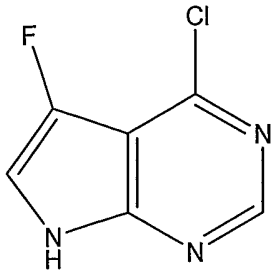
nucleoside would include α-*D*-ribo, β-*L*-ribo, α-*L*-ribo, β-*D*-xylo, α-*D*-xylo, β-*L*-xylo, α-*L*-xylo, β-*D*-lyxo, α-*D*-lyxo, β-*L*-lyxo, α-*L*-lyxo, β-*D*-arabino, α-*D*-arabino, β-*L*-arabino, α-*L*-arabino, β-*D*-deoxy, α-*D*-deoxy, β-*L*-deoxy, α-*L*-deoxy, β-*D*-dideoxy, α-*D*-dideoxy, β-*L*-dideoxy, α-*L*-dideoxy nucleoside analogs of compound **6** as well as 2', 3', 4' and 5' substituted nucleosides including alkoxy, halo and alkyl substituted nucleosides, *e.g*, 2' and 3'-alkoxy and 2' and 3'-fluoro nucleosides. These are prepared by reaction of compound **2** and the corresponding α-*D*-ribo, β-*L*-ribo, α-*L*-ribo, β-*D*-xylo, α-*D*-xylo, β-*L*-xylo, α-*L*-xylo, β-*D*-lyxo, α-*D*-lyxo, β-*L*-lyxo, α-*L*-lyxo, β-*D*-arabino, α-*D*-arabino, β-*L*-arabino, α-*L*-arabino, β-*D*-deoxy, α-*D*-deoxy, β-*L*-deoxy, α-*L*-deoxy, β-*D*-dideoxy, α-*D*-dideoxy, β-*L*-dideoxy, α-*L*-dideoxy sugar derivative or 2', 3', 4' and 5' derivative of these sugars.

[0016] 5-fluorotubercidin was prepared as described in the detailed examples below. 5-fluorotubercidin as well as 5-iodotubercidin and tubercidin were evaluated for cellular cytotoxicity using an MTT assay against Huh-7 liver cells, normal mouse spleen cells stimulated with Con A (a T-cell mitogen), and normal mouse spleen stimulated with LPS (a B-cell mitogen). No significant toxicity was observed for these compounds (IC50 > 200 μM) in these cells. However in fibroblast cells tubercidin was toxic at 12 μM whereas no toxicity was observed for 5-fluorotubercidin in concentrations of up to 200 μM. In L1210 cells 5-iodotubercidin showed activity at > 10 μM, tubercidin at 2-3 μM and 5-fluorotubercidin at 1 μM. Thus increased anti-proliferative toxicity of 5-fluorotubercidin **(7)** compared to tubercidin was observed against L-1210 tumor cells, while toxicity in fibroblast cells was reduced. While we do not wish to be bound by theory, this suggests 5-fluorotubercidin might be less cytotoxic than tubercidin and thus have a greater therapeutic index for antitumor indications as compared to tubercidin.

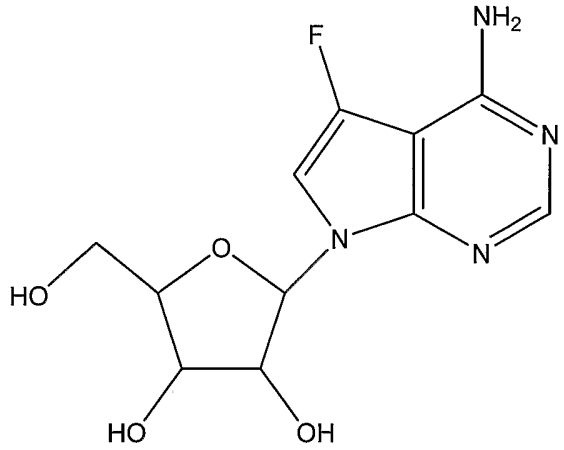
[0017] 5-fluorotubercidin was also compared to 5-iodotubercidin and tubercidin for their ability to inhibit bacterial transcription/translation as well as antibacterial activity against E. *coli* and S. *aureus*. No antibacterial activity observed at concentrations up to 100 μM.

[0018] 5-halogenated pyrrolo[2,3-*d*]pyrimidine nucleosides can be prepared by a direct halogenation of an appropriately protected tubercidin. For example, direct bromination of tubercidin with NBS in DMF gives the 5-bromotubercidin, while the use of NBS in a KOAc-buffered medium gives 6-bromotubercidin (Bergstrom, D. E.et al. *Nucleic Acids Research,* **8**: 6213-6219, 1980). In a similar fashion, the 5-iodo and 5-chloro analogs can also be prepared. However, the direct fluorination of pyrrolo[2,3-*d*]pyrimidine nucleosides resulted in no reaction or cleavage of the glycosidic bond, as determined with a variety of electrophilic fluorinating conditions.

[0019] In the present invention, the 5-fluoro analogues of pyrrolo[2,3-*d*]pyrimidine, *e.g.*, 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine and 5-fluorotubercidin (4- amino-5-fluoro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine nucleoside), *i.e.*:



4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine,



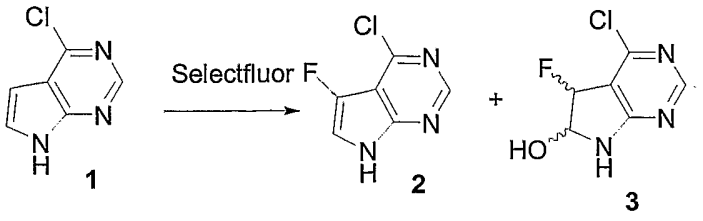
4-amino-5-fluoro-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine nucleoside (5-fluorotubercidin),

were prepared by the following synthesis protocol. The 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine heterocycle was first prepared and coupled to an appropriately substituted ribose. 4-Chloro-pyrrolo[2,3-*d*]pyrimidine (**1**) was chosen as starting material. Other 4-substitutent groups might also be used including 4-halo, 4-oxo, 4-protected amino and 4-alkyl where halo includes bromo, chloro, fluoro and iodo and alkyl is C1 to C6 alkyl. While we do not wish to be bound by any particularly theory, the 4-chloro moiety is preferred to facilitate the electrophilic fluorination process. Generally, electrophilic fluorination of heterocycles is carried out using the highly reactive fluorine gas or acetyl hypofluorites (Erian, A. W. *Journal of Heterocyclic Chemistry,* **38**: 793-808, 2001). In the present invention, we used mild fluorination conditions directly on the electron-rich 5-position of 4-chloro-pyrrolo[2,3-*d*]pyrimidine **1** using commercially available reagents. A variety of conditions and electrophilic fluorinating reagents were tested (NFSI; 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor, Aldrich Chemical Co.) in acetonitrile, DMF and

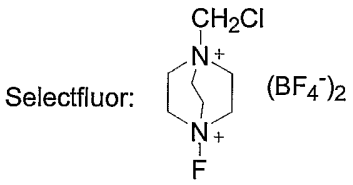
dichloromethane at various temperatures) (Singh, et al., *Acc Chem Res.*, **37**: 31-44, 2004. Lal, G. S.; et al. *Chemical Reviews (Washington, D. C.),* **96**: 1737-1755, 1996. Taylor, S. D.; et al. *Tetrahedron,* **55**: 12431-12477, 1999. Banks, R. E. *Journal of Fluorine Chemistry,* **87**: 1-17, 1998). Selectfluor/acetonitrile was used in the following syntheses.

[0020] Treatment of compound **1** with Selectfluor in wet acetonitrile for 4 h at room temperature, gave compound **3** as a (9:1) mixture of diastereoisomers as the main products (Scheme 1). Alternatively, a similar reaction in scrupulously dried acetonitrile in the presence of activated molecular sieves gave product **2.** A one-pot hydro-fluorination-elimination was effected by adding TFA or AcOH, as both of these acids had been successfully used in pyrrolo[2,3-*d*]pyrimidine 5,6-dehydrations (Migawa, M. T. et al. *Synth. Commun.,* **26**: 3317-3322, 1996). However, there was no reaction with either acid at room temperature. Increasing the reaction temperature to 70-80 °C for 30 min in the presence of TFA resulted in a mixture of starting material and both products **2** and **3**. Prolonged heating resulted in decomposition. In contrast, the dehydration was successful using AcOH at elevated temperature, as treatment of **1** with Selectfluor in the presence of AcOH at 70-80 °C up to approximately 14 hours affected smooth conversion to the desired compound **2**. Furthermore, this process was amenable to scale-up, as compound **2** was obtained in a 59% yield on a 5 gram reaction.

Scheme 1. Electrophilic Fluorination of 5-Chloropyrrolo[2,3-*d*]pyrimidine.

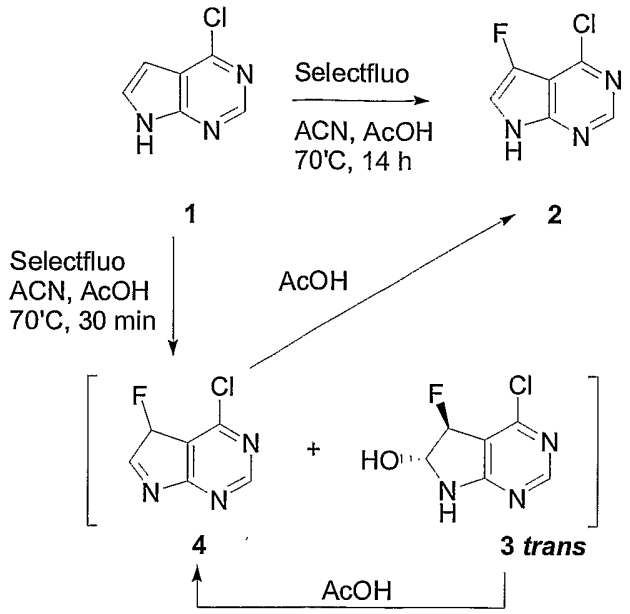


Solvent	Additions	Condition	Result
DCM	none	23°C / 14 h	no reaction
DMF	none	23°C / 14 h	no reaction
CH_3CN	H_2O	23°C / 4 h	**1** + **3** (major)
CH_3CN	3Å MS	23°C / 14 h	**1** + **2**
CH_3CN	AcOH	70°C / 14 h	**2**



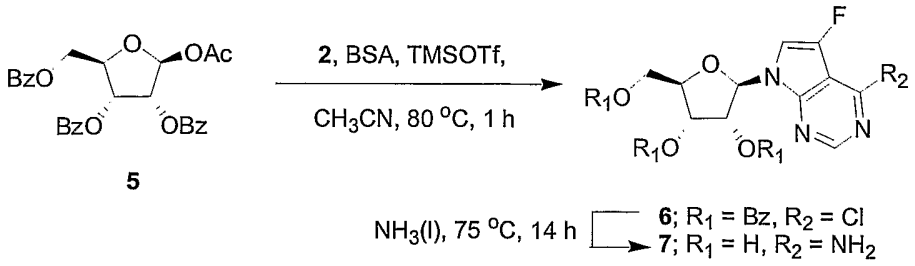
[0021] After about 30 min at 70 °C, most of starting material **1** was consumed and one LC/MS peak (retention time 2.6 min) corresponding to the mass of compound **2** was observed. After 2 h, an additional peak (retention time 2.7 min) was formed having a mass spectrum identical to that of the peak at 2.6 min. After running the reaction overnight, only the peak at 2.7 min remained. While not wishing to be bound by any particularly theory, it is suspected that the 5-H tautomer **4** (Scheme 2) might be the peak observed at 2.6 min, and is formed as an intermediate between compounds **2** and **3**.

Scheme 2. Mechanism of Electrophilic Fluorination .



[0022] In order to more fully explore the hypothesis, the major diastereomer (9:1 ratio) of compound **3** was isolated by silica gel chromatography. The structure was determined to have the hydroxy and fluoro groups in a *trans* orientation (**3 *trans)*** since the coupling constant (close to 0 Hz) of the 5,6-hydrogens for the major isomer is less than the one for minor isomer (5.8Hz). The dehydration of compound **3 *trans*** was investigated by ^{1}H NMR in presence of AcOD-d_3 in Acetonitrile-d_3 at 70°C (Figure 3) and by ^{19}F NMR (not shown). It can be clearly observed that after heating for 2.5 h at 70 °C, tautomer **4** is present as a significant product (together with small amount of tautomer **2** and compound **3 *trans*)**. After further heating, compound **2** was observed as the major product. 5-fluorotubercidin, i.e., 4-amino-5-fluoro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine) (**7**) was synthesized from 4-chloro-5-fluoropyrrolo[2,3-*d*]pyrimidine (**2**) using a modified Vorbruggen procedure to give 4-chloro-5-fluoro-7-(2,3,5-tri-*O*-benoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6), in a 65% yield (Vorbrueggen, H., *Acc. Chem. Res.*, **28**: 509-520, 1995. Migawa, M. T. et al. *Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7-11*; pp MEDI-250, 1997). Treatment of compound **6** with ammonia (l) in dioxane gave the 5-fluorotubercidin, compound **7**.

Scheme 3. Synthesis of 5-Fluorotubercidin.



EXPERIMENTAL EXAMPLES

[0023] General: NMR spectra were recorded on a 300 M Hz Bruker. Silica gel 60 from EM Science was used for purification. Compound **1** was purchased from Toronto Research Chemicals. Selectfluor was purchased from Aldrich.

[0024] Coupled Bacterial Transcription/Translation Assay. The DNA template, pBest Luc ™ (Promega), is a plasmid containing a reporter gene for firefly luciferase fused to a strong *tac* promoter and ribosome binding site. Messenger RNA from 1 μg pBestLuc was transcribed and translated in *E. coli* S30 bacterial extract in the presence or absence of test compound. Compounds were tested in a black 96 well microtiter plate with an assay volume of 35 μL. Each test well contained: 5 μL test compound, 13 μL S30 premix (Promega), 4 μL 10X complete amino acid mix (1 mM each), 5 μL *E. coli* S30 extract and 8 μL of 0.125 μg/μL pBest Luc™. The transcription/translation reaction was incubated for 35 minutes at 37°C followed by detection of functional luciferase with the addition of 30 μL LucLite™ (Packard). Light output was quantitated on a Packard TopCount.

[0025] Minimum Inhibitory Concentrations (MICs). The assays are carried out in 150 μL volume in duplicate in 96-well clear flat-bottom plates. The bacterial suspension from an overnight culture growth in appropriate medium is added to a solution of test compound in 2.5% DMSO in water. Final bacterial inoculum is approximately 10^2-10^3 CFU/well. The percentage growth of the bacteria in test wells relative to that observed for a control wells containing no compound is determined by measuring absorbance at 595 nm (A_{595}) after 20-24 h at 37°C. The MIC is determined as a range of concentration where complete inhibition of growth is observed at the higher concentration and bacterial cells are viable at the lower concentration. Both ampicillin and tetracycline are used as antibiotic positive controls in each screening assay for *E. coli* (ATCC 25922) and *S. aureus* (ATCC13709)

[0026] MTT Assays. MTT proliferation assays were purchased as kits from Promega and were run according to the manufacturer's protocol.

Example 1

4-Chloro-5-fluoro-7H-pyrrolo[2,3-*d*]pyrimidine (2).

[0027] 4-Chloro-7*H*-pyrrolopyrimidine **1** (5 g, 32.7 mmol) and Selectfluor (17.35 g, 49 mmol) were placed in a round bottom flask, followed by the addition of dry acetonitrile (250 mL) and AcOH (50 mL). The solution was then heated at 70 °C for 14 h under N_2. After cooling to room temperature, the solvent was removed *in vacuo* and co-evaporated with toluene (50 mL × 2). The solid was dissolved in a mixture of DCM : EtOAc (1:1) and filtered through a pad of silica gel which was thoroughly washed. The combined washings were evaporated. And the crude product was then subjected to column chromatography with DCM : EtOAc (4:1) to give 3.3 g (59% yield) of **2** as a white solid. ^{1}H NMR (DMSO-d_6) δ 7.73 (s, 1H), 8.64 (s, 1H), 12.5 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 105.3 (d, J = 14.3 Hz), 111.0 (d, J = 25.5 Hz), 139.6 (d, J = 244.5 Hz), 146.7 (d, J = 1.5 Hz), 148.5 (d, J = 3.8 Hz), 151.0; ^{19}F NMR (DMSO-d_6) δ –170.7 (d, J = 1.6 Hz): Mass spectroscopy (MS) measured for $C_6H_3ClFN_3$ (M+H): 172.0, observed: 172.0.

Example 2

4-Chloro-5-fluoro-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-6-ol (3).

[0028] 4-Chloro-7*H*-pyrrolopyrimidine **1** (10 mg, 0.065 mmol) and Selectfluor (115 mg, 0.33 mmol) were placed in a round bottom flask, followed by the addition of acetonitrile (1 mL) and two drops of water. The mixture was then stirred at room temperature for 4 hours. After evaporation, the crude product was purified by column chromatography using DCM : MeOH (98:2) to give 5 mg brown solid. ^{1}H NMR (CD_3CN) δ 8.19 (d, 1H, J = 1.9 Hz), 5.51 (d, 1H, J = 53.9 Hz), 5.19 (d, 1H, J = 21.9 Hz); ^{19}F NMR (CD_3CN) δ –177.5 (ddd, J = 1.9, 21.6, 53.6 Hz): MS calcd for $C_6H_5ClFN_3O$ (M+H): 190.0, observed: 190.1.

Example 3

4-Chloro-5-fluoro-7-(2,3,5,-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6).

[0029] *N,O*-Bis(trimethylsilyl)acetamide (BSA, 0.16 mL, 0.64 mmol) was added to a stirred suspension of 4-chloro-5-fluoro-7H-pyrrolo[2,3-*d*]pyrimidine (0.1 g, 0.58 mmol) in dry acetonitrile (4 mL). After stirring at room temperature for 10 min, 1-*O*-acetyl-2,3,5,-tri-*O*-benzoyl-β-D-ribofuranose (0.322 g, 0.64 mmol) was added, followed by the addition of trimethylsily trifluoromethanesulfonate (0.115 mL, 0.64 mmol). The reaction was stirred at room

temperature for 15 min after which it was transferred to a preheated oil bath at 80°C. After stirring for 1 hour at 80°C, the reaction was cooled to room temperature and diluted with EtOAc (25 mL). The organic phase was then sequentially washed with saturated. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated to provide the crude nucleoside. Purification by column chromatography (SiO_2, 10–25% EtOAc in hexanes) provided protected nucleoside (**6**) as a white foam (232 mg, 65%). ^1H NMR ($CDCl_3$) δ: 8.63 (s, 1H), 8.11 (d, 2H, J = 7.1 Hz), 8.01 (d, 2H, J = 7.1 Hz), 7.91 (d, 2H, J = 7.1 Hz), 7.65–7.33 (m, 9H), 7.17 (d, 1H, J = 2.6 Hz), 6.69 (dd, 1H J = 6.2, 1.1 Hz), 6.09 (m, 2H), 4.87 (dd, 1H, J = 12.1, 3.0 Hz), 4.78 (dd, 1H, J = 7.2, 3.7 Hz), 4.67 (dd, 1H, J = 12.1, 3.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 166.1, 165.4, 165.1, 151.9, 151.1, 147.1, 143.7-140.3 (C-F-coupling), 133.8, 133.7, 133.6, 129.8, 129.8, 129.7, 129.3, 128.7, 128.7, 128.6, 128.5, 128.4, 109.5-109.1 (C-C-F-coupling), 108.3-108.1 (C-C-F-coupling), 86.3, 80.6, 73.9, 71.4, 63.6. MS (M+H): 616.1, observed: 616.1.

Example 5

5-Fluorotuberidicin (7).

[0030] 4-Chloro-5-fluoro-7-(2,3,5,-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **6** (230 mg, 0.42 mmol) was dissolved in dioxane (3 mL) and liquid ammonia (8 – 10 mL). The reaction was sealed in a steel bomb and heated in an oil bath at 75°C for 14 hours. The reaction was then cooled and the solvent evaporated to provide the crude nucleoside. Purification by column chromatography (SiO_2, 5–15% MeOH in $CHCl_3$) provided 5-fluorotubericidin (**7**) as a white solid (77 mg, 73%). ^1H NMR (DMSO-d_6) δ: 8.06 (s, 1H), 7.34 (d, 1H, J = 2 Hz), 6.99 (s, br, 2H), 6.06 (dd, 1H, J = 6.1, 1.7 Hz), 5.28 (d, 1H, J = 6.4 Hz), 5.10 (m, 2H), 4.31 (m, 1H), 4.05 (m, 1H), 3.85 (m, 1H), 3.7–3.14 (m, 2H). ^{13}C NMR (DMSO-d_6) δ: 156.3, 152.6, 146.7, 144.7-141.4 (C-F coupling), 105.0-104.6 (C-C-F coupling), 93.1-92.9 (C-C-F coupling), 86.4, 84.9, 73.7, 70.4, 61.5. ^{19}F NMR (DMSO-d_6) δ: -167.73. MS $(M+H)^+$: 285.1, observed: 284.9.

Example 6

MTT Toxicity Assay

[0031] The MTT cell proliferation assay was used to test for cell toxicity (see van de Loosdrecht, A. A.; Beelen, R. H.; Ossenkoppele, G. J.; Broekhoven, M. G.; Langenhuijsen, M. M. *J. Immunol. Methods* 1994, *174*, 311-320). An assay kit was purchased from American Type Culture Collection (Manassas, VA, USA), and treatment of cells and the specific assay protocol was carried out according to the manufacturer's recommendations. The MTT cell proliferation

assay measures cell viability and growth by the reduction of tetrazolium salts. In the assay a yellow tetrazolium salt is reduced in metabolically active cells to form purple formazan crystals that are solubilized by the addition of detergent. The color was quantified by spectrophotometric means. For each cell type a linear relationship between cell number and absorbance was established, enabling quantification of changes of proliferation.

[0032] The MTT toxicity data for 5-fluorotubercidin in various cell lines is shown in the below Table 1. 5-Fluorotubercidin shows a greater toxicity (IC_{50} = 1 μM) to the rapidly proliferating L1210 cell line as compared to the other cell lines tested.

	MTT Assay IC_{50} (μM)				
Cell Line	Huh-7 Liver	Mouse Spleen Con-A Stimulated	Mouse Spleen LPS Stimulated	L-1210 Lymphoblastoid	Fibroblast
5-Fluorotubercidin	>200	>200	>200	1	>200

[0033] Evaluations of cellular cytotoxicity were carried out in an MTT assay (Promega) against Huh-7 liver cells, normal mouse spleen cells stimulated with Con A (a T-cell mitogen), normal mouse spleen stimulated with LPS (a B-cell mitogen), L-1210 lymphoblastoid cells and fibroblast cells. The 5-fluorotubercidin compound was non toxic (IC_{50} > 200μM) for Huh-7 liver epithelial cells, normal mouse spleen cells stimulated with Con A (a T-cell mitogen), and normal mouse spleen stimulated with LPS (a B-cell mitogen). However, cell toxicity activity of 5-fluorotubercidin was observed against L-1210 cells (>10 μM for 5-iodotubercidin, 2-3 μM for tubercidin and 1 μM for 5-fluorotubercidin). Toxicity in fibroblast cells was observed at concentrations above 12 μM for tubercidin, while no toxicity was observed for 5-fluorotubercidin in concentration of up to 200 μM.

Example 7

Cyquant Proliferation Assay

[0034] Cyquant cell proliferation assay was used to test compounds for cell toxicity. In the assay, 5-fluorotubercidin showed inhibitory active as measured by retardation of cell growth of both HeLa cell line and human lung cancer cell line H460 (wild-type-p53) at concentrations ranging from 200 nM to 2000 nM.

[0035] An assay kit (Molecular Probes #C-7026) was used for treatment of cells with 5-fluorotubercidin. Treatment of cells and the specific assay protocol was carried out according to the manufacturer's recommendations. The Cyquant assay utilizes green fluorescent dye (CyQUANT GR) that exhibits strong fluorescence enhancement when bound to cellular nucleic

acids. After treatment, cells were frozen, thawed and lysed in a buffer containing the CyQUANT GR dye. Fluorescence was measured directly and gives a linear representation of relative cell number.

[0036] Cyquant proliferation data was measured for both HeLa cells and H460 cells. In the HeLa cell proliferation assay, at the start of the assay baseline fluorescence measured 10000 RFU. At concentrations below 200 nM no cell growth retardation activity was noted. At a concentration of 200 nM at 25 minutes the baseline 10000 RFU value was maintain whereas at 50 minutes the cells begin to proliferate and fluorescence increase to 17000 RFU and at 75 minutes to 22500 RFU. At a concentration of 1000 nM, at 25 minutes the fluorescence was increased slightly to 11000 whereas at 50 minutes fluorescence had decrease to 8000 RFU and at 75 minutes decreased again to 4500 RFU. At a concentration of 5000 nM, at 25 minutes fluorescence decrease to 4500 RFU and stayed at this value for both the 50 minutes and 75 minutes time points.

[0037] In the H460 cell proliferation assay, at the start of the assay baseline fluorescence measured 5000 RFU. At a concentration of 200 nM from the 5000 baseline fluorescence at 25 minutes fluorescence increase slightly to 7500 RFU increase again at 50 minutes to 8000 RFU and again at 75 minutes to 11000 RFU. At a concentration of 1000 nM, at 25 minutes the fluorescence increase to 5500 whereas at 50 minutes fluorescence had decrease 4000 RFU and at 75 minutes to 3500 RFU. At a concentration of 5000 nM, at 25 minutes fluorescence decrease to 4500 RFU. At 50 minutes it decrease to 2000 RFU and at 75 minutes to under 2000 RFU.

[0038] In both assays, 5-fluorotubercidin showed inhibitory active as measured by retardation of cell growth starting at the 200 nM concentrations. Retardation of growth of both HeLa and H460 tumor cell lines also was seen at concentrations of the 1000 nM and 2000 nM.

[0039] The present invention provides methods of making compound suitable for use in pharmaceutical compositions that comprise a therapeutically effective amount of one or more compounds described herein, their pharmaceutically acceptable salts, prodrugs or derivatives and a pharmaceutically acceptable carrier.

[0040] Such pharmaceutical compositions can be used as nucleoside antimetabolites in cancer chemotherapy or treatment of benign or malignant abnormal tissue growth. Such pharmaceutical compositions can be used to treat or prevent a variety of neoplastic diseases such as, solid tumors, hematological malignancy, leukemia, colorectal cancer, benign or malignant breast cancer, uterine cancer, uterine leiomyomas, ovarian cancer, endometrial cancer, polycystic ovary syndrome, endometrial polyps, prostate cancer, prostatic hypertrophy, pituitary cancer,

adenomyosis, adenocarcinomas, meningioma, melanoma, bone cancer, multiple myeloma, CNS cancers, such as glioma or astroblastoma, and other benign or malignant abnormal tissue growth.

[0041] The term "pharmaceutically acceptable salt, prodrug or derivative," as used herein, related to any pharmaceutically acceptable salt, ester, ether, salt of an ester, solvate, such as ethanolate, or other derivative of a compound of the present invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an active metabolite or residue thereof. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (*e.g.*, by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (*e.g.*, the brain or lymphatic system).

[0042] The compounds of the present invention can be used in the form of salts derived from pharmaceutically or physiologically acceptable acids or bases. These salts include, but are not limited to, the following salts with inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid and, as the case may be, such organic acids as acetic acid, oxalic acid, succinic acid, and maleic acid. Other salts include salts with alkali metals or alkaline earth metals, such as sodium, potassium, calcium or magnesium in the form of esters, carbamates and other conventional "pro-drug" forms, which, when administered in such form, convert to the active moiety *in vivo*.

[0043] When the compounds are employed for the above utilities, they may be combined with one or more pharmaceutically acceptable carriers, for example, solvents, diluents and the like, and may be administered orally in such forms as tablets, capsules, dispersible powders, granules, or suspensions containing, for example, from about 0.05 to 5% of suspending agent, syrups containing, for example, from about 10 to 50% of sugar, and elixirs containing, for example, from about 20 to 50% ethanol, and the like, or parenterally in the form of sterile injectable solutions or suspensions containing from about 0.05 to 5% suspending agent in an isotonic medium. Such pharmaceutical preparations may contain, for example, from about 25 to about 90% of the active ingredient in combination with the carrier, more usually between about 5% and 60% by weight.

[0044] Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the pyrrolo[2,3-*d*]pyrimidine compounds (*see, e.g., Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA 18th ed., 1990, incorporated herein

by reference). The pharmaceutical compositions generally comprise the pyrrolo[2,3-*d*]pyrimidine compounds in a form suitable for administration to a patient. The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0045] The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration and the severity of the condition being treated. However, in general, satisfactory results are obtained when the compounds of the invention are administered at a daily dosage of from about 0.5 to about 500 mg/kg of animal body weight, preferably given in divided doses two to four times a day, or in a sustained release form. For most large mammals, the total daily dosage is from about 1 to 100 mg, preferably from about 2 to 80 mg. Dosage forms suitable for internal use comprise from about 0.5 to 500 mg of the active compound in intimate admixture with a solid or liquid pharmaceutically acceptable carrier. This dosage regimen may be adjusted to provide the optimal therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[0046] These active compounds may be administered orally as well as by intravenous, intramuscular, or subcutaneous routes. Solid carriers include starch, lactose, dicalcium phosphate, microcrystalline cellulose, sucrose and kaolin, while liquid carriers include sterile water, polyethylene glycols, non-ionic surfactants and edible oils such as corn, peanut and sesame oils, as are appropriate to the nature of the active ingredient and the particular form of administration desired. Adjuvant customarily employed in the preparation of pharmaceutical compositions may be advantageously included, such as flavoring agents, coloring agents, preserving agents, and antioxidants, for example, vitamin E, ascorbic acid, BHT and BHA.

[0047] The preferred pharmaceutical compositions from the standpoint of ease of preparation and administration are solid compositions, particularly tablets and hard-filled or liquid-filled capsules. Oral administration of the compounds is preferred.

[0048] These active compounds may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid, polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0049] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringe ability exits. It must be stable under conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacterial and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oil.

[0050] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included.

[0051] The disclosures of each patent, patent application and publication cited or described in this document are hereby incorporated herein by reference, in their entirety for all purposes.

[0052] Those skilled in the art will appreciate that numerous changes and modifications can be made to the embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

What is Claimed:

1. A method comprising the steps of:

providing a 4-substituted pyrrolo[2,3-*d*]pyrimidine; and

reacting said 4-substituted pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-substituted 5-fluoro-pyrrolo[2,3-*d*]pyrimidine.

2. The method of claim 1, wherein said 4-substituted pyrrolo[2,3-*d*]pyrimidine is 4-halo-pyrrolo[2,3-*d*]pyrimidine.

3. The method of claim 2, wherein said 4-halo-pyrrolo[2,3-*d*]pyrimidine is 4-chloro-pyrrolo[2,3-*d*]pyrimidine.

4. The method of claim 1, further comprising reacting said 4-substituted pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid.

5. The method of claim 1, wherein said electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).

6. The method of claim 1, wherein said reaction of said 4-substituted pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C.

7. The method of claim 1, wherein said reaction with said electrophilic fluorinating reagent occurs for a time period of approximately 2 to approximately 14 hours.

8. The method of claim 1 wherein said 4-substituted pyrrolo[2,3-*d*]pyrimidine comprises 4-halo, 4-oxo, 4-alkyl or 4-protected amino pyrrolo[2,3-*d*]pyrimidine.

9. A method comprising the steps of:

providing 4-halo-pyrrolo[2,3-*d*]pyrimidine; and

reacting said 4-halo-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-halo-5-fluoropyrrolo[2,3-*d*]pyrimidine.

10. The method of claim 9, further comprising reacting said 4-halo-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid.

11. The method of claim 9, wherein said electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).

12. The method of claim 9, wherein said reaction of said 4-halo-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C.

13. The method of claim 9, wherein said reaction with said electrophilic fluorinating reagent occurs for a time period of approximately 2 to approximately 14 hours.

14. A method comprising the steps of:

providing 4-chloro-pyrrolo[2,3-*d*]pyrimidine; and

reacting said 4-chloro-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-chloro-5-fluoropyrrolo[2,3-*d*]pyrimidine.

15. The method of claim 14, further comprising reacting said 4-chloro-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid.

16. The method of claim 14, wherein said electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).

17. The method of claim 14, wherein said reaction of said 4-chloro-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C.

18. The method of claim 14, wherein said reaction with said electrophilic fluorinating reagent occurs for a time period of approximately 2 to approximately 14 hours.

19. A method comprising the steps of:

providing 4-substituted pyrrolo[2,3-*d*]pyrimidine;

reacting said 4-substituted pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-substituted 5-fluoro-pyrrolo[2,3-*d*]pyrimidine;

reacting said 4-substituted 5-fluoro-pyrrolo[2,3-*d*]pyrimidine with a sugar derivative to form 4-substituted 5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside; and

reacting said 4-substituted 5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside with ammonia to form 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside.

20. The method of claim 19, wherein said 4-substituted pyrrolo[2,3-*d*]pyrimidine is 4-halo-pyrrolo[2,3-*d*]pyrimidine.

21. The method of claim 19, wherein said 4-substituted pyrrolo[2,3-*d*]pyrimidine is 4-chloro-pyrrolo[2,3-*d*]pyrimidine.

22. The method of claim 19, further comprising reacting said 4-substituted pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid.

23. The method of claim 19, wherein said electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).

24. The method of claim 19, wherein said reaction of said 4-substituted pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C.

25. The method of claim 19, wherein said reaction with said electrophilic fluorinating reagent occurs for a time period of approximately 2 to 14 hours.

26. The method of claim 19, wherein said sugar derivative is a β-*D*, β-*L*, α-*D* or α-*L* form of a ribo, xylo, lyxo and arabino sugar and further including 2', 3', 4' or 5' substituted derivatives of said sugar derivative.

27. The method of claim 19, wherein said sugar derivative is a D-ribofuranose derivative.

28. The method of claim 27, wherein said D-ribofuranose derivative is 1-*O*-acetyl-2,3,5,-tri-*O*-benzoyl-β-D-ribofuranose.

29. The method of claim 19 wherein said 4-substituted pyrrolo[2,3-*d*]pyrimidine comprises 4-halo, 4-oxo, 4-alkyl or 4-protected amino pyrrolo[2,3-*d*]pyrimidine.

30. A method comprising the steps of:

providing 4-halo-pyrrolo[2,3-*d*]pyrimidine;

reacting said 4-halo-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-halo-5-fluoropyrrolo[2,3-*d*]pyrimidine;

reacting said 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine with a sugar derivative to form 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside; and

reacting said 4-halo-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside with ammonia to form 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside.

31. The method of claim 30, wherein said 4-halo-pyrrolo[2,3-*d*]pyrimidine is 4-chloro-pyrrolo [2,3-*d*]pyrimidine.

32. The method of claim 30, further comprising reacting said 4-halo-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid.

33. The method of claim 30, wherein said electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).

34. The method of claim 30, wherein said reaction of said 4-halo-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C.

35. The method of claim 30, wherein said reaction with said electrophilic fluorinating reagent occurs for a time period of approximately 2 to 14 hours.

36. The method of claim 30, wherein said sugar derivative is a a β-*D*, β-*L*, α-*D* or α-*L* form of a ribo, xylo, lyxo and arabino sugar and further including 2', 3', 4' or 5' substituted derivatives of said sugar derivative.

37. The method of claim 30, wherein said sugar derivative is a D-ribofuranose derivative.

38. The method of claim 37, wherein said D-ribofuranose derivative is 1-*O*-acetyl-2,3,5,-tri-*O*-benzoyl-β-D-ribofuranose.

39. A method comprising the steps of:

providing 4-chloro-pyrrolo[2,3-*d*]pyrimidine;

reacting said 4-chloro-pyrrolo[2,3-*d*]pyrimidine with an electrophilic fluorinating reagent to form 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine;

reacting said 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine with a sugar derivative to form 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside; and

reacting said 4-chloro-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside with ammonia to form 4-amino-5-fluoro-pyrrolo[2,3-*d*]pyrimidine nucleoside.

40. The method of claim 39, further comprising reacting said 4-chloro-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent in the presence of acetonitrile and acetic acid.

41. The method of claim 39, wherein said electrophilic fluorinating reagent is 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).

42. The method of claim 39, wherein said reaction of said 4-chloro-pyrrolo[2,3-*d*]pyrimidine with said electrophilic fluorinating reagent is effected at a temperature that is approximately 70°C.

43. The method of claim 39, wherein said reaction with said electrophilic fluorinating reagent occurs for a time period of approximately 2 to 14 hours.

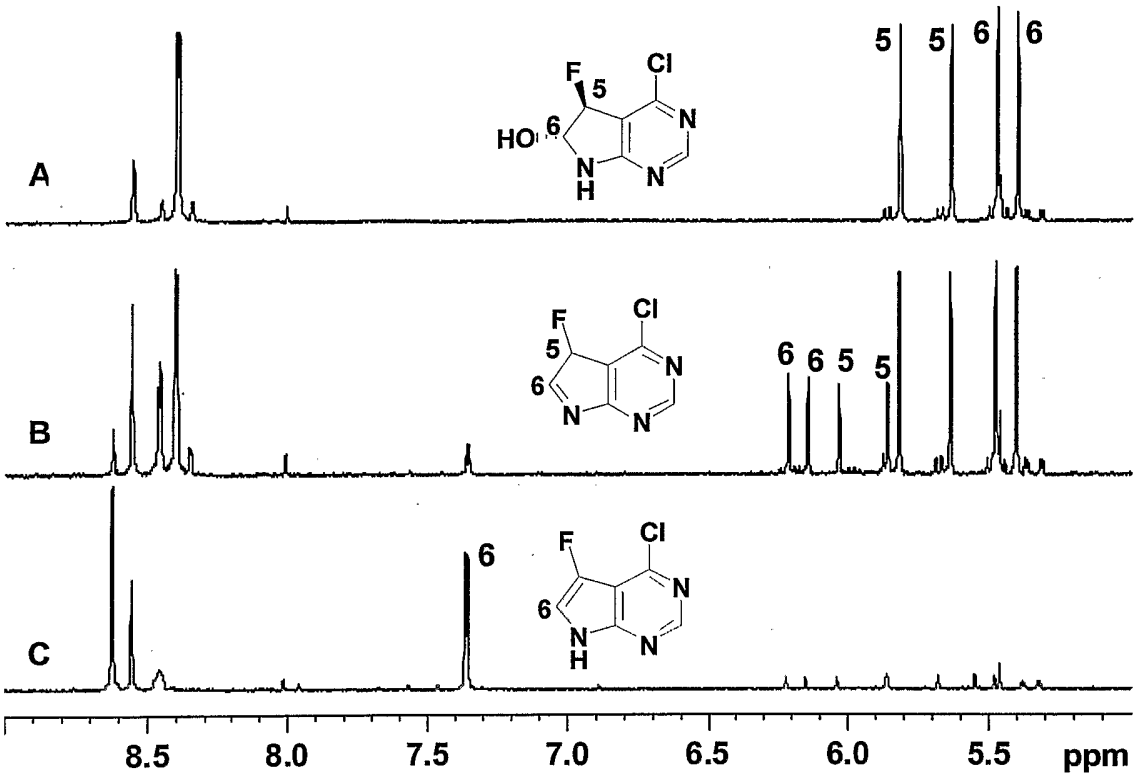
44. The method of claim 39, wherein said sugar derivative is a β-*D*, β-*L*, α-*D* or α-*L* form of a ribo, xylo, lyxo and arabino sugar and further including 2', 3', 4' or 5' substituted derivatives of said sugar derivative.

45. The method of claim 39, wherein said sugar derivative is a D-ribofuranose derivative.

46. The method of claim 45, wherein said D-ribofuranose derivative is 1-*O*-acetyl-2,3,5,-tri-*O*-benzoyl-β-D-ribofuranose.

Figure 1

^{1}H NMR Spectra of Dehydration



A) 25°C, 5 min; B) 70°C, 2.5h; C) 70°C, 14h.