# Adenosine Kinase Inhibitors. 1. Synthesis, Enzyme Inhibition, and Antiseizure Activity of 5-Iodotubercidin Analogues

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Adenosine receptor agonists produce a wide variety of therapeutically useful pharmacologies. However, to date they have failed to undergo successful clinical development due to dose-limiting side effects. Adenosine kinase inhibitors (AKIs) represent an alternative strategy, since AKIs may raise local adenosine levels in a more site- and event-specific manner and thereby elicit the desired pharmacology with a greater therapeutic window. Starting with 5-iodotubercidin ($IC_{50} = 0.026\ \mu M$) and 5′-amino-5′-deoxyadenosine ($IC_{50} = 0.17\ \mu M$) as lead inhibitors of the isolated human AK, a variety of pyrrolo[2,3-*d*]pyrimidine nucleoside analogues were designed and prepared by coupling 5-substituted-4-chloropyrrolo[2,3-*d*]pyrimidine bases with ribose analogues using the sodium salt-mediated glycosylation procedure. 5′-Amino-5′-deoxy analogues of 5-bromo- and 5-iodotubercidins were found to be the most potent AKIs reported to date ($IC_{50}$s $< 0.001\ \mu M$). Several potent AKIs were shown to exhibit anticonvulsant activity in the rat maximal electric shock (MES) induced seizure assay.

## Introduction

Adenosine receptor activation has long been known to produce profound and diverse pharmacologies, including effects on hemodynamics, CNS activity, cellular functions, and metabolism.[1,2] Consequently, compounds that activate adenosine receptors, such as adenosine and adenosine receptor agonists, represent potential therapeutic agents for a variety of diseases including hypertension, epilepsy, pain, diabetes, and inflammation.[3] However, adenosine receptor agonists exhibit a relatively narrow therapeutic index due to simultaneous production of undesired pharmacologies and have therefore failed to undergo successful clinical development.

Over the past decade, we have pursued alternative strategies that would harness the potential therapeutic benefits of adenosine receptor activation while minimizing the side effects.[4−11] The approach entails the use of agents termed "adenosine regulating agents (ARAs)" that affect the production or metabolism of adenosine such that extracellular adenosine is elevated in a relatively site- and event-specific manner. The hypothesized specificity of action is based on the principle that extracellular adenosine is produced primarily from intracellular breakdown of ATP.[12] Net ATP breakdown occurs in cells that are subjected to hypoxia or cellular stress (e.g.: neurons at seizure foci) such that repletion of ATP stores is not possible at a rate comparable with energy utilization. Agents that divert ATP breakdown toward production of adenosine, or agents that decrease the metabolism of adenosine, should therefore enhance extracellular adenosine levels specifically within hypoxic regions of tissues and have little effect in normoxic regions.

One potential ARA target is adenosine kinase (AK). AK is a cytosolic enzyme that catalyzes the conversion of adenosine to AMP. AK activation represents the major clearance route of adenosine and is partly responsible for its extremely short plasma half-life (<1 s).[13] Inhibition of AK results in increased intracellular adenosine which passes out of the cell via passive diffusion or via nucleoside transporter(s) to activate nearby cell-surface adenosine receptors.[14] Thus, AK inhibition could represent an alternative mechanism for activation of adenosine receptors and production of adenosine-associated pharmacologies.

Our initial studies of AKIs focused on their potential use as antiseizure agents.[15] Adenosine is postulated to be an endogenous anticonvulsant, i.e., a substance produced in the brain to control seizure activity.[16] Support for this hypothesis comes from studies showing adenosine receptor antagonists increase the severity and duration of seizures and from studies showing adenosine levels rise dramatically during seizures.[17−19] Consistent with this hypothesis are studies that show adenosine agonists inhibit seizures in a dose-dependent manner.[19] We and others have found that AKIs inhibit seizure activity,[15,20] and we found that these agents to show fewer side effects than adenosine receptor agonists.[15]

At the outset of our studies, three compounds were reported to be potent AK inhibitors: namely, 5-iodotubercidin,[21] (**1a**), 5′-deoxy-5-iodotubercidin,[22,23] (**1b**), and 5′-amino-5′-deoxyadenosine[24] (**2**) (Chart 1), which were found in our laboratory to be potent inhibitors of the isolated human AK with $IC_{50}$s 0.026, 0.009, and 0.17 $\mu$M, respectively. These compounds, however, exhibited significant therapeutic limitations. For example, **1a** is suspected to be a cytotoxic agent via 5′-phosphorylation by intracellular kinases,[15] while **1b** is reported to produce significant CNS side effects and muscle flaccidity.[26] Compound **2**, on the other hand, exhibited weak in vivo activity and a short half-life[15] due to deamination by adenosine deaminase.[27] As part of an effort to find

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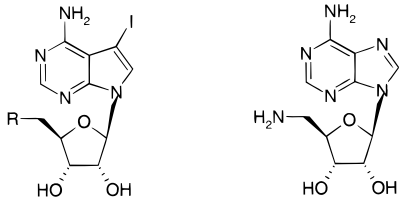
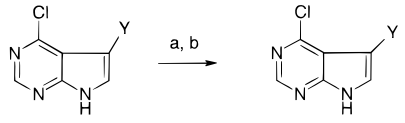
**Chart 1**



**1a** R = OH
**1b** R = H

**2**

**Scheme 1**[a]



**3a** Y = I
**3b** Y = Br

**4a** Y = $CH_3$
**4b** Y = $SCH_3$
**4c** Y = COOEt

[a] (a) THF, *n*-BuLi, −78 °C; (b) MeI, MeSSMe or Cl-COOEt.

highly potent AKIs with better in vivo properties, we synthesized a series of tubercidin analogues and evaluated their SAR for AK inhibition and antiseizure activity.

## Chemistry

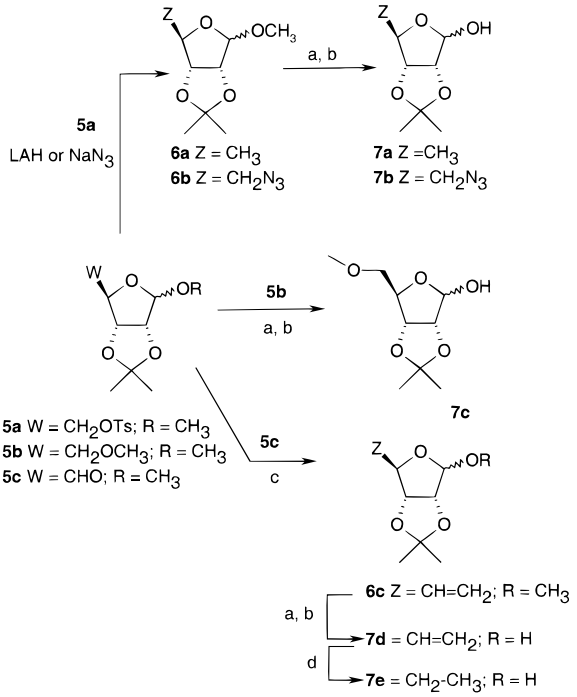
The general strategy for the synthesis of compounds listed in Table 1 involved glycosylation of 5-substituted 4-chloropyrrolo[2,3-*d*]pyrimidine bases with various 2,3-*O*-isopropylidene-protected α-ribofuranosyl chlorides followed by deprotection. The resulting 4-chloropyrrolo[2,3-*d*]pyrimidine nucleosides were condensed with amines to obtain the desired final products.

5-Substituted 4-chloro[2,3-*d*]pyrimidine bases **3a**,[28] **3b**,[29] and **4a**[30] were prepared by the literature procedures, whereas **4b,c** were prepared as shown in Scheme 1 and used in the glycosylation step. Lithium halogen exchange of **3b**[28] followed by treatment with dimethyl disulfide gave 4-chloro-5-methylthiopyrrolo[2,3-*d*]pyrimidine (**4b**) in 52% yield, whereas treatment with ethyl chloroformate gave 4-chloro-5-ethoxycarbonylpyrrolo-[2,3-*d*]pyrimidine (**4c**) in 72% yield.

The carbohydrate precursors **7a**−**e** used in the glycosylation step were synthesized as shown in Scheme 2. Reduction of methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-D-ribofuranoside (**5a**) with lithium aluminum hydride provided **6a**[30] in 70% yield. When heated with $NaN_3$ in DMF, **5a** gave the corresponding 5-azido analogue **6b** in 85% yield. Efforts to convert **6a**,**b** to **7a**,**b**, respectively, under a variety of mild acidic conditions without simultaneously losing the isopropylidene protecting group were unsuccessful. Therefore, **6a**,**b** were subjected to complete deprotection using 0.1 N $H_2SO_4$ at 80−90 °C. The resulting ribose analogues were treated with 2,2-dimethoxypropane in DMF in the presence of *p*-toluenesulfonic acid to reintroduce the isopropylidene protecting group giving the desired **7a**,**b**, respectively, in moderate overall yields. Similarly, 2,3-*O*-isopropylidene-5-*O*-methyl-D-ribofuranose (**7c**) was prepared from **5b**[30] by manipulating the protecting groups as described for the conversion of **6a**,**b** to **7a**,**b**, respectively.

Synthesis of sugar intermediates with extended carbon chains **7d**,**e** was accomplished starting with known

**Scheme 2**[a]



[a] (a) 0.1 N $H_2SO_4$; (b) 2,2-dimethoxypropane/*p*-toluenesulfonic acid; (c) $Ph_3P^+CH_3I^-$/*n*-BuLi; (d) $H_2$, Pd/C.

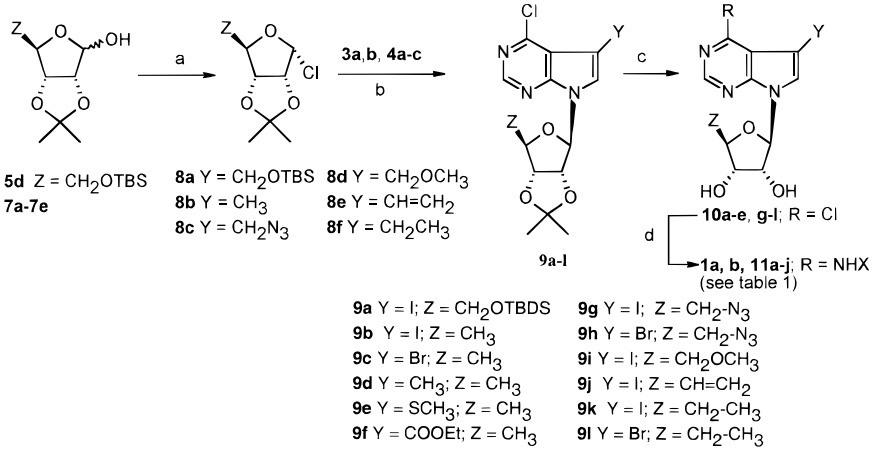
aldehyde **5c**,[31] which was reacted with methyltriphenylphosphonium ylide to give **6c** in 80% yield. Acid-catalyzed hydrolysis of **6c** followed by reprotection with 2,2-dimethoxypropane gave **7d** in 75% yield. Subsequent hydrogenation of **7d** using Pd/C gave **7e** in quantitative yield.

The α-chloro sugars needed for the glycosylation of various heterocycles were prepared as shown in Scheme 3 via a literature method[32] described for the synthesis of 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-ribofuranosyl chloride (**8a**). Reaction of **5d**, **7a**−**e** with $CCl_4$ and hexamethylphosphorus triamide at −78 °C in anhydrous tetrahydrofuran gave the corresponding α-chloro sugars **8a**−**f**. Under these reaction conditions **5d** is reported to give exclusively the α-chloro anomer **8a** which epimerizes to its *β*-chloro anomer at 90 °C over a period of 30 h.[32]

The stereochemical assignments of the ribosyl halides were made by $^1$H NMR spectroscopy. An aliquot from the chlorination of **7a** was evaporated quickly under nitrogen, dissolved in DMSO-$d_6$, and an $^1$H NMR spectrum was recorded. A doublet at 6.12 ppm ($J_{1-2}$ = 4.1 Hz) is characteristic of a *β*-anomeric proton of α-chloro sugar **8b**. The NMR sample was heated at 60−65 °C, and the anomerization was followed by the slow disappearance of the original doublet for the anomeric proton and simultaneous formation of a singlet at 6.0 ppm which is characteristic of the anomeric proton of a *β*-chloro sugar.[32]

Although anomerization of **8a** to the corresponding *β*-chloro anomer is reported to take place at 90 °C over a period of 30 h, the α-ribofuranosyl chlorides **8b**−**f** were found to undergo considerable anomerization at ambient temperatures in the reaction mixture. The half-life of **8b**,**c** ranged between 1 and 2 h in DMSO-$d_6$ and 12−36 h in $CDCl_3$. An improvement in the stability of **8a** was noticed when the chlorination mixture was passed

**Scheme 3**[a]




[a] (a) $CCl_4$/toluene, HMPT, −20 °C; (b) NaH/$CH_3CN$; (c) 70% TFA; (d) $XNH_2$.

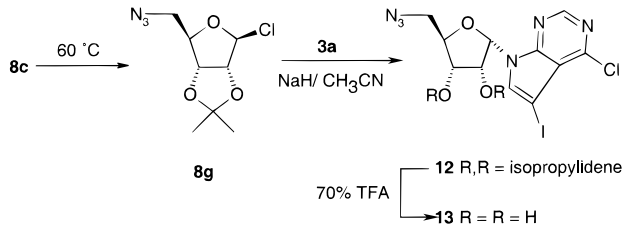
through a short silica gel column to remove unreacted HMPT and the reaction byproduct HMPA.

Glycosylations of various pyrrolopyrimidine bases were carried out via the sodium salt procedure,[33] as shown in Scheme 3. Sodium salts of the pyrrolopyrimidine bases **3a**,**b** and **4a**−**c** were generated using NaH in anhydrous acetonitrile at 0 °C, and the preformed α-chloro sugar solutions (**8a**−**f**) were directly cannulated into the reaction mixture. The reaction mixtures were stirred overnight at room temperature to give **9a**−**l** in moderate yields (25−45%), after silica gel chromatography. The glycosylation products, with the exception of **9f** (Y = COOEt; Z = $CH_3$), were deprotected with 70% trifluoroacetic acid (TFA) at room temperature to give C5-substituted-4-chloropyrrolo[2,3-*d*]pyrimidine nucleosides **10a**−**e**,**g**−**l**. Efforts to deprotect **9f** under a variety of mild acidic conditions resulted in deglycosylation. However, successful deprotection was achieved by first aminating **9f** with methanolic ammonia and then treating with 70% TFA giving **11c** in an overall 55% yield. Amination of the intermediates **10a**−**e**,**g**−**l** with methanolic ammonia or methylamine at 90−120 °C gave the final products **1a**−**c** and **11a**,**b**,**d**−**j** in moderate isolated yields.

The stereochemical assignments of the glycosylation products were made by comparative $^1$H NMR spectroscopic data with those reported for similar pyrrolo[2,3-*d*]pyrimidine nucleosides.[33] The anomeric proton of the completely deprotected product(s) (**10a**−**l**) shows a doublet between 5.9 and 6.3 ppm with a coupling constant of 5−6 Hz. However, a smaller coupling constant ($J \leq 3$ Hz) for the anomeric proton of the isopropylidene protected intermediates **9a**−**l** was observed. In addition, the two methyl signals for the isopropylidene protecting group of β-nucleosides are characteristically farther apart from each other (0.2−0.25 ppm) than those of α-nucleosides (0.1−0.15 ppm).[33]

Further proof in support of the β-configuration comes from the NOE experiments on a few isopropylidene protected nucleosides. For example, irradiation of the *endo*-methyl signal of the isopropylidene protecting group of **9b** resulted in the enhancement of the C1′-H and C4′-H signals which is characteristic of a β-nucleoside since both C1′- and C4′-protons are on the α-face of the sugar moiety. To obtain a definitive proof of anomeric configuration, an α-nucleoside **13** was synthesized as shown in Scheme 4. The α-chloro sugar **8c** was stirred at 60 °C until it was converted to β-chloro sugar **8g** and reacted with the sodium salt of **4a** to give **12** after chromatography. Deprotection of **12** with 70% TFA provided **13** in an overall 22% yield. The $^1$H NMR spectrum of **13** was found to be characteristically different from the corresponding β-anomer **10g**. As is expected for α-nucleosides, the anomeric proton signal **13** appears at a slightly lower field (6.65 ppm) with a larger coupling constant ($J = 7.92$ Hz) compared to that of the corresponding β-anomer **10g**. In addition, the methyl signals of the isopropylidene protecting group of **12** are characteristically closer together (0.1−0.15 ppm) compared to those of its β-anomer **9g**. Last, in the NOE experiment, irradiation of the *endo*-methyl signal of **12** resulted in the signal enhancement for only the C4′-H indicating that it is the only proton on the α-face of the sugar moiety.

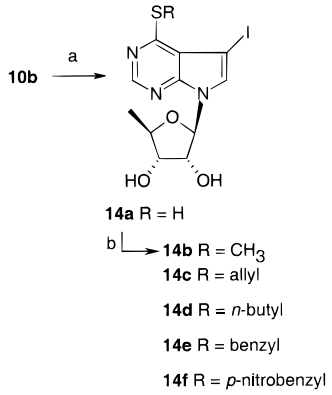
**Scheme 4**




Various C4-mercapto derivatives of **1b** selected for SAR studies were synthesized in a two-step procedure, as shown in Scheme 5. Reaction of **10b** with thiourea in refluxing ethanol gave the thione **14a**, which was subsequently alkylated with various electrophiles to give the corresponding C4-alkylthio or C4-aralkylthio compounds **14b**−**f**.

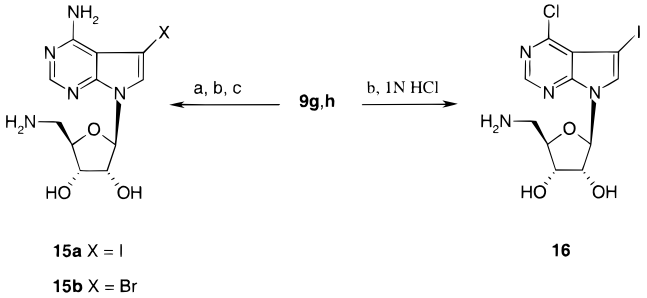
Synthesis of various 5′-amino-5′-deoxynucleosides was achieved as shown in Scheme 6. Initial efforts to activate the 5′-OH group of isopropylidene protected derivative **1a** to its *p*-toluenesulfonyl ester resulted in an intractable mixture of products presumably due to the formation of N1−C5′ cyclonucleoside. A similar observation was made when the same intermediate was subjected to Mitsunobu reaction conditions. Therefore, 5′-azido sugar **7b** was used to obtain the corresponding 5′-azido nucleosides **9g**,**h** which were aminated with methanolic ammonia to give the corresponding C4-$NH_2$ intermedi-

**Scheme 5**[a]




[a] (a) Thiourea/EtOH, reflux; (b) $NH_4OH$/R-I(Br).

**Scheme 6**[a]



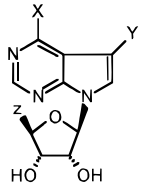

[a] (a) $NH_3$/MeOH; (b) $PPh_3/NH_4OH$; (c) 70% TFA.

ates. These intermediates were subjected to reduction using $PPh_3$ and $NH_4OH$, followed by TFA catalyzed deprotection to give **15a**,**b**, respectively, in low overall yields. The 5′-amino-4-chloro derivative **16** was obtained by reacting **9g** with $PPh_3/NH_4OH$, followed by deprotection with 1 N HCl solution, as shown in Scheme 6.

## Results and Discussion

**SAR of Enzyme Inhibition**. Compounds listed in Table 1 were evaluated as inhibitors of the recombinant human AK, and the $IC_{50}$s were determined by a radiochemical assay by a slightly modified literature method.[34] The purpose of the enzyme inhibition study was to evaluate the effect of modifications at the C4-, C5-, and C5′-positions of tubercidin on the AKI activity. Initial efforts were focused on the C5-position since substitution with an iodo group converts a modest AK substrate tubercidin ($K_M = 10\ \mu M$) into a potent AK inhibitor (**1a**, $IC_{50} = 0.026\ \mu M$). The results indicate that a halogen atom such as Cl, Br, or I at the C5-position of pyrrolopyrimidine nucleosides is essential for potent AKI activity. Moreover, the inhibitory potency increases with increasing size and electron density of the halogen atom. The large atomic size of I appears to provide a hydrophobic interaction with the active site of the enzyme. To examine the effect of hydrophobic and electron-donating groups on the AKI activity, groups such as $CH_3$ and $SCH_3$ were introduced at the C5-position. These compounds (**11a**,**b**), however, were found to be poor inhibitors of the enzyme. The loss of AKI potency shown by **11a** may be attributed to the smaller size of the $CH_3$ group capable of providing only weak hydrophobic interactions compared to any of the halogen atoms. Although $SCH_3$ is an electron-rich group, the decreased potency of **11b** appears to be due to poor fitting of the group in the active site. Therefore, the potent AKI activity exhibited by **1a**,**c**,**d** appears to due to the combined effect of lipophilicity and electronegativity of the C5-substituent.

**Table 1.** AK Inhibitor SAR




| compd | X | Y | Z | AK $IC_{50}$ ($\mu$M)[a] |
|---|---|---|---|---|
| **1a**[21] | $NH_2$ | I | $CH_2OH$ | 0.026 |
| **1b**[23] | $NH_2$ | I | $CH_3$ | 0.009 |
| **1c**[23] | $NH_2$ | Br | $CH_3$ | 0.04 |
| **1d**[21] | $NH_2$ | Br | $CH_2OH$ | 0.12 |
| **1e**[21] | $NH_2$ | Cl | $CH_2OH$ | 0.21 |
| **10a**[21] | Cl | I | $CH_2OH$ | 0.024 |
| **10b** | Cl | I | $CH_3$ | 0.003 |
| **10c** | Cl | Br | $CH_3$ | 0.05 |
| **10d** | Cl | $CH_3$ | $CH_3$ | 0.535 |
| **10e** | Cl | $SCH_3$ | $CH_3$ | 0.07 |
| **10g** | Cl | I | $CH_2N_3$ | 0.009 |
| **10h** | Cl | Br | $CH_2N_3$ | 0.1 |
| **10i** | Cl | I | $CH_2OCH_3$ | 0.45 |
| **11a** | $NH_2$ | $CH_3$ | $CH_3$ | 16.5 |
| **11b** | $NH_2$ | $SCH_3$ | $CH_3$ | 1.35 |
| **11c** | $NH_2$ | COOEt | $CH_3$ | 1.0 |
| **11d** | $NH_2$ | I | $CH_2N_3$ | 0.035 |
| **11e** | $NH_2$ | Br | $CH_2N_3$ | 0.063 |
| **11f** | $NH_2$ | I | $CH_2OCH_3$ | 1.2 |
| **11g** | $NH_2$ | I | $CH{=}CH_2$ | 0.1 |
| **11h** | $NH_2$ | I | $CH_2CH_3$ | 0.06 |
| **11i** | $NH_2$ | Br | $CH_2CH_3$ | 0.5 |
| **11j** | NH-$CH_3$ | I | $CH_2OH$ | 1.2 |
| **14a** | SH | I | $CH_3$ | 6.5 |
| **14b** | $SCH_3$ | I | $CH_3$ | 0.045 |
| **14c** | *S*-allyl | I | $CH_3$ | 2.8 |
| **14d** | *S*-*n*-$C_4H_9$ | I | $CH_3$ | 1.0 |
| **14e** | *S*-benzyl | I | $CH_3$ | 1.0 |
| **14f** | *S*-*p*-nitrobenzyl | I | $CH_3$ | 2.0 |
| **15a** | $NH_2$ | I | $CH_2NH_2$ | 0.0006 |
| **15b** | $NH_2$ | Br | $CH_2NH_2$ | 0.0002 |
| **15c**[36] | $NH_2$ | H | $CH_2NH_2$ | 13.0 |
| **16** | Cl | I | $CH_2NH_2$ | 0.0001 |
| **17**[35] | $NH_2$ | CN | $CH_2OH$ | 0.31 |
| **18**[36] | $NH_2$ | CN | $CH_3$ | 0.31 |
| **19**[35] | $NH_2$ | $CONH_2$ | $CH_2OH$ | 0.47 |
| **20**[36] | $NH_2$ | $CONH_2$ | $CH_3$ | 0.46 |

[a] Enzyme inhibition assays were performed on recombinant human AK. $IC_{50}$ values are results of a single experiment.

Efforts were also directed toward evaluating the effect of electron-withdrawing groups at the C5-position. For example, **11c**, a C5-COOEt-substituted compound, inhibited the enzyme with an $IC_{50} = 1.0\ \mu M$. Other naturally occurring tubercidin analogues with similar electron-withdrawing groups at the C5-position, such as toyocamycin[35] (**17**, C5-cyanotubercidin, $IC_{50} = 0.31\ \mu M$) and sangivamycin[35] (**19**, C5-carboxamidotubercidin, $IC_{50} = 0.47\ \mu M$) showed moderate AKI activity. Furthermore, their 5′-deoxy analogues **18**[36] and **20**[37] were also found to be moderate inhibitors of the enzyme suggesting that strong electron-withdrawing groups at the C5-position are relatively less-tolerated.

Further SAR studies were directed toward evaluating the effect of various groups at the C4-position of the tubercidin molecule. The results indicate that compounds with a C4-$NH_2$ group in conjunction with Br or

I at the C5-position exhibit potent enzyme inhibitory activity. However, a dramatic decrease in potency was observed for the corresponding C4-$NHCH_3$ analogue **11j**. The moderate to high potency exhibited by the C4-chloro analogues **10a**−**e,g**−**i**, on the other hand, indicates that Cl is also tolerated at the C4-position, especially when there is I or Br at the C5-position. Although, the C4-$SCH_3$-substituted compound **14b** ($IC_{50}$ = 0.045 $\mu$M) showed potent AKI activity, the corresponding C4-*S*-allyl (**14c**), -*S*-butyl (**14d**), -*S*-benzyl (**14e**), and -*S*-*p*-nitrobenzyl (**14f**) substituted compounds were found to be poor inhibitors of the enzyme.

The potent inhibitory activity exhibited by compounds containing $NH_2$, Cl, or $SCH_3$ at the C4-position indicates that the enzyme accommodates both hydrophilic as well as hydrophobic groups at this site. It is conceivable that hydrophobic groups such as C4-Cl and C4-$SCH_3$ occupy an empty space with or without hydrophobic interaction within the enzyme active site, whereas a C4-$NH_2$ group is held by a hydrogen bonding with the enzyme presumably through water molecules. The bulkier alkylthio groups of **14c**−**f**, on the other hand, are too large to fit into the presumed empty pocket thereby resulting in poor AKI activity.

Adenosine is a substrate of AK with a $K_M$ = 0.280 $\mu$M which also acts as an inhibitor of the enzyme at higher concentration ($IC_{50}$ = 1 mM).[27] When the 5′-OH of adenosine is replaced with another strong hydrogen-bond-forming group such as $NH_2$, the resulting molecule **2** was found to potently inhibit of the enzyme ($IC_{50}$ = 0.12 $\mu$M).[27] This indicates a strong interaction between the active site and the 5′-$NH_2$ of **2**. Therefore, it was envisioned that introduction of an $NH_2$ group at the C5′-position of **1a**,**b** would further enhance their AKI potency. Accordingly, 5′-amino-5′-deoxy compounds **15a,b** and **16** were prepared and were found to show >10-fold enhancement in the AKI potency ($IC_{50} \leq$ 0.0006 $\mu$M). However, 5′-amino-5′-deoxytubercidin[36] **(15c)** ($IC_{50}$ = 13 $\mu$M), a compound without a halogen at the C5-position, was found to be a very weak AKI, reiterating the importance of a halogen atom at the C-5 position for the tubercidin molecules to potently inhibit AK.

Potent AKI activity of **1b** indicates that in addition to hydrogen-bonding OH and $NH_2$ groups, the enzyme may accommodate at the C5′-position a non-hydrogen-bonding group such as H. To further evaluate this site, C5′-homologated analogues of **1b** such as **11g**−**i** were prepared. A considerable loss in AKI potency of these compounds ($IC_{50}$s 0.06−0.5 $\mu$M) suggests that hydrophobic groups larger than H at C5′-position are less-tolerated by the enzyme. Introduction of an azide group, a dipolar function with a net neutral charge, at the C5′-position also resulted in potent AKIs (**10g**,**h**, **11d**−**e**, $IC_{50}$ 0.009−0.1 $\mu$M) which is attributed to a possible electrostatic interaction between an azide group and the enzyme. However, C5′-*O*-methyl analogue **11f** was found to be a poor inhibitor ($IC_{50}$ = 1.2 $\mu$M) suggesting poor fitting of C5′-$OCH_3$ group in the active site.

Recently, the 3-D structure of human AK was determined by X-ray crystallography.[38] Since the coordinates were generated using a X-ray crystal structure of an AK−adenosine complex instead of an AK−inhibitor complex, the information was not used in interpreting the SAR of compounds disclosed in this report.

**Table 2.** Antiseizure Activity in Rat MES Seizure Model

| compd | $ED_{50}$ (mg/kg)[a] | compd | $ED_{50}$ (mg/kg)[a] |
|---|---|---|---|
| **1a**[15] | 6.0 | **15a**[15] | 7.1 |
| **1b**[15] | 0.3 | **15b** | 4.2 |
| **1c** | <3.7[b] | | |

[a] Tested at 1 h following ip administration. [b] **1c** inhibited MES by 83% at 3.7 mg/kg.

**SAR of Antiseizure Activity.** Several AKIs were evaluated in the standard maximum electroshock seizure (MES) model (Table 2). The anticonvulsant effect observed with 5-iodotubercidin (**1a**) was in agreement with activity observed against bicuculline-induced seizures following local administration of the AKI into the prepiriform cortex.[20] The most potent compound, **1b**, had an $ED_{50}$ of 0.3 mg/kg, whereas the others showed potencies within the range of 4.2−7.1 mg/kg. The rank order of anticonvulsant activity in vivo did not strongly correlate with the order of potency in the enzyme inhibition assay in vitro, suggesting that variations in the molecular substituents result in substantial changes in pharmacokinetic properties such as clearance rate, brain penetration, or accessibility to the intracellular enzyme. For example, compounds **15a**,**b**, C5′-amino analogues of **1b**,**c**, showed lower potencies in vivo despite greater potencies against the enzyme; this may be a result of decreased blood−brain barrier penetration by the C5′-amino analogues which are less hydrophobic than their corresponding 5′-deoxy analogues. It is also possible that these compounds may act by additional mechanisms that may influence in vivo activity. Such mechanisms would likely be related to adenosine, since the in vivo activities of these compounds are antagonized by administration of adenosine receptor antagonists.[39] The involvement of direct agonist activity on A1 or A2 adenosine receptors has been ruled out as these compounds show little affinity for the receptors.[15] Similarly the compounds show no detectable activity on adenosine deaminase (ADA) or on adenosine monophosphate deaminase (AMPDA) and little or no affinity to the nitrobenzylthioinosine binding site associated with a transport mechanism.[15] However undefined transport systems could be modulated by the compounds, perhaps influencing the accumulation of extracellular adenosine.

Side effects observed for the AKIs included decreased locomotor activity, hypothermia, and muscular flaccidity. These effects were generally mild at the anticonvulsant $ED_{50}$s.

## Conclusion

In summary, we have explored the effect of various substitutions at the C4-, C5-, and C5′-positions of tubercidin on AK inhibition and have described new AKIs with increased potency. We have also demonstrated anticonvulsant activity of several of these compounds against MES induced seizures in rats. It is clear from these studies that a halogen atom such as Cl, Br, or I at the C5-position and either a Cl, $NH_2$, or $SCH_3$ group at the C4-position of the pyrrolo[2,3-*d*]-pyrimidine ring system are essential for tubercidin analogues to potently inhibit AK. The C5′-position appears to accommodate both hydrophobic and hydrophilic groups, with the amino group at this position resulting in three of the most potent AK inhibitors

described to date (**15a**,**b** and **16)**. In vivo, the potencies of these compounds were similar to that of **1a**, and further modifications will be required to enhance in vivo properties. The ability of potent AK inhibitors to inhibit maximal seizures induced by electroshock indicates that these compounds are highly efficacious as anticonvulsants and suggests that inhibition of AK is a viable alternative to adenosine receptor agonists as a purinergic approach to inhibition of seizure activity.

## Experimental Section

$^1$H and $^{13}$C NMR spectra were obtained using a Varian Gemini-200 spectrophotometer at 200 MHz. NOE experiments were conducted on a Bruker AM-500 spectrophotometer at 500 MHz by NuMega Resonance Labs, Inc., San Diego, CA. The chemical shifts are expressed in $\delta$ units with respect to tetramethylsilane ($\delta$ 0.00) as an internal standard. The infrared spectra were obtained using a Perkin-Elmer-1310 infrared spectrophotometer using KBr pellets and the values are expressed as cm$^{-1}$. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel, GHLF 250-$\mu$m plates. Silica gel, 230−400 mesh (E. Merck), was used for the column chromatography. Elemental analyses were determined by Robertson Microlit Laboratories, Madison, NJ.

**4-Chloro-5-methylthiopyrrolo[2,3-*d*]pyrimidine (4b).** To a solution of 5-bromo-4-chloropyrrolo[2,3-*d*]pyrimidine[29] (**3b**; 2.67 g, 11.5 mmol) in dry THF (50 mL) at −78 °C was added, dropwise, to a solution of *n*-BuLi (10 mL of 2.5 M soln in THF) at a rate so as to maintain the reaction temperature below −60 °C. After an additional 30 min of stirring, the reaction mixture was treated with a solution of dimethyl disulfide (4 mL, 42.5 mmol) in THF (10 mL) over 30 min and the mixture was then allowed to warm to room temperature overnight. The reaction was quenched with a saturated solution of $NH_4Cl$ (25 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$), evaporated, and the crude product was crystallized from boiling ethanol to give **4b** as a white crystalline solid (1.2 g, 52%): mp 166−167 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.81 (s, 1H), 8.35 (s, 1H), 11.0 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 17.95, 105.62, 114.24, 127.82, 149.52, 149.84, 151.50. Anal. ($C_7H_6ClN_3S$) C, H, N.

**4-Chloro-5-ethoxycarbonylpyrrolo[2,3-*d*]pyrimidine (4c).** To a solution of the dianion of **4b** (10 mmol) generated by the above procedure was added a solution of ethyl chloroformate (1.15 mL, 11 mmol) in THF (5 mL) over a period of 20 min. The reaction mixture was allowed to warm to room temperature overnight and worked up as described for **4b**. The resulting crude product was crystallized from ethanol to give **4c** as an off-white crystalline solid (1.6 g, 72%): mp 140−141 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.25−1.49 (t, 3H), 4.2−4.35 (q, 2H), 8.38 (d, 1H), 8.7 (s, 1H), 11.6 (br s, 1H). Anal. ($C_9H_8ClN_3O_2$) C, H, N.

**5-Azido-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranose (7b).** A mixture of methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-D-ribofuranoside **(5a)**[30] (17.9 g, 0.05 mol), $NaN_3$ (6.5 g, 0.1 mol) and anhydrous DMF (75 mL) was heated at 90 °C for 12 h. The solvent was evaporated under high vacuum and the residue partitioned between water (200 mL) and ethyl acetate (200 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to give methyl 5-azido-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranoside (**6b**) as a colorless syrup (9.7 g, 85%): $^1$H NMR ($CDCl_3$) $\delta$ 1.25 and 1.45 (2s, 6H), 3.30 (m, 2H), 3.35 (s, 3H), 4.27 (t, 1H), 4.59 (m, 2H), 4.95 (s, 1H); IR (KBr) 2170 cm$^{-1}$. A suspension of **6b** in 0.1 N $H_2SO_4$ (200 mL) was heated in an oil bath at 85 °C for 4 h. The reaction mixture was cooled in an ice bath, and the pH was adjusted to ca. 7.5 using Dowex 1×8-50, $OH^-$ resin. The resin was removed by filtration, the filtrate evaporated under reduced pressure, and the residual oil kept under high vacuum for 3 h. To the dried product was added dry DMF (60 mL), 2,2-dimethoxypropane (20 mL) and *p*-toluenesulfonic acid (100 mg). After stirring at room-temperature overnight, the volatile portions were removed under high vacuum and the residue was purified by flash chromatography (10% methanol in $CH_2Cl_2$) to give **7b** as a colorless oil (7.28 g, 80%): $^1$H NMR (CDCl3) $\delta$ 1.25 and 1.45 (2s, 6H), 3.45 (m, 2H), 4.30 (t, 1H), 4.61 (m, 2H), 5.45 (d, 1H).

**2,3-*O*-Isopropylidene-5-*O*-methyl-D-ribofuranose (7c).** A solution of 5-*O*-methyl-D-ribofuranose[30] (12.5 g, 84.5 mmol) in dry DMF (185 mL), 2,2-dimethoxypropane (20 mL) and *p*-toluenesulfonic acid (250 mg) was stirred overnight at room temperature. The reaction mixture was worked up as described for **7b** to give **7c** as an oily product (12.6 g, 82%): $^1$H NMR ($CDCl_3$) $\delta$ 1.25 and 1.45 (2s, 6H), 3.37 (s, 3H), 3.52 (m, 2H), 4.30 (m, 1H), 4.45 and 4.70 (2d, 2H), 5.2 (br s, 1H).

**Methyl 5,6-Didehydro-5,6-dideoxy-2,3-*O*-isopropylidene-D-allofuranoside (6c).** Methyltriphenylphosphonium iodide (29.0 g, 81 mmol) was added in portions to a suspension of *t*-BuOK (9.36 g, 83.6 mmol) in anhydrous ether (300 mL). The bright yellow colored solution was stirred at room temperature for 2 h and then treated with a solution of **5c**[31] (8.0 g, 39.6 mmol) in anhydrous ether (75 mL). After stirring overnight at room temperature, the reaction mixture was filtered to remove the solid and the solid was washed with ether. The filtrate and the washings were combined, concentrated under reduced pressure and the residue was chromatographed on silica gel (10% ethyl acetate in hexanes) to give **6c** as a colorless oil (6.5 g, 80%): $^1$H NMR ($CDCl_3$) $\delta$ 1.32 and 1.56 (2s, 6H), 3.37 (s, 3H), 4.64 (m, 3H), 5.10 (s, 1H), 5.17 (dt, 1H,), 5.92 (m, 1H).

**5,6-Didehydro-5,6-dideoxy-2,3-*O*-isopropylidene-D-allofuranose (7d).** Compound **6c** (6.0 g, 30 mmol) was subjected to complete deprotection, and the resulting syrupy product was reacted with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid as described for **7b** to give **7d** as a colorless oil (4.2 g, 75%): $^1$H NMR (DMSO-$d_6$) $\delta$ 1.23 and 1.45 (2s, 6H), 4.52 (m, 3H), 5.10 (br d, 1H), 5.25 (m, 2H), 6.05 (m, 1H), 6.6 (d, 1H, exchangeable with $D_2O$).

**5,6-Dideoxy-2,3-*O*-isopropylidene-D-allofuranose (7e).** A degassed solution of **7d** (5.6 g, 30 mmol) in methanol (50 mL) was treated with Pd on carbon (10%, 200 mg) and subjected to hydrogenation in a Parr apparatus under 50 psi of $H_2$ for 72 h. The catalyst was removed by filtration and the filtrate evaporated to give **7e** as a colorless oil (5.5 g, 95%): $^1$H NMR (DMSO-$d_6$) $\delta$ 0.93 (t, 3H), 1.25 and 1.38 (2s, 6H), 1.52 (m, 2H), 3.85 (t, 1H), 4.52 (m, 2H), 5.19 (d, 1H), 6.42 (d, 1H, exchangeable with $D_2O$).

**5-Deoxy-2,3-*O*-isopropylidene-α-D-ribofuranosyl Chloride (8b) and Its Anomerization. 8b** was made by the previously described procedure.[40] An aliquot of **8b** was anomerized to the corresponding $\beta$-chloro sugar by heating the NMR sample in a water bath at 55−60 °C for 3 h: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 and 1.40 (2s, 6H), 1.46 (d, 3H), 4.5 (q, 1H), 4.62 (d, 1H), 5.03 (d, 1H), 6.12 (s, 1H).

**5-Azido-5-deoxy-2,3-*O*-isopropylidene-α-D-ribofuranosyl Chloride (8c)**. Compound **8c** was prepared from **7b** by the procedure[40] described for **8b**, and was used without isolation: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 and 1.55 (2s, 6H), 3.65 (m, 2H), 4.35 (m, 1H), 4.72 and 5.91 (2m, 2H), 6.46 (d, $J$ = 4.6 Hz, 1H). The NMR sample was kept in a water bath at 60 °C for 2 h to allow anomerization of the α-chloro sugar to the $\beta$-chloro sugar (**8g**): $^1$H NMR (DMSO-$d_6$) $\delta$ 1.31 and 1.42 (2s, 6H), 3.60 (m, 2H), 4.42 (m, 1H), 4.61(d, 1H), 5.15 (d, 1H), 6.48 (s, 1H).

**4-Chloro-5-iodo-7-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9a).** To an ice-cold solution of **3a**[28] (7.0 g, 25 mmol) in dry acetonitrile (50 mL) was added NaH (80% dispersion in oil, 0.75 g, 25 mmol) in small portions, and stirred for 30 min. A solution of **8a**[32] (prepared from 15.25 g of **5a**, 50 mmol) was cannulated into the reaction mixture and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to give a residue which was stirred with ethyl acetate (50 mL) and filtered. The filtrate was evaporated and the crude product chromatographed on silica

gel (25% ethyl acetate in hexanes) to provide **9a** as a glassy product (5.9 g, 42%): $^1$H NMR (DMSO-$d_6$) $\delta$ 0.85 (m, 15H), 1.33 and 1.55 (2s, 6H), 3.75 (m, 2H), 4.25 (m, 1H), 4.82 (m, 1H), 5.25 (m, 1H), 6.32 (d, $J$ = 2.6 Hz, 1H), 8.10 (s, 1H), 8.70 (s, 1H).

**4-Chloro-5-iodo-7-(5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9b).** Glycosylation of **3a** with **8b** as described for **9a** gave **9b** as a glassy solid in 43% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.27 (d, $J$ = 6.5 Hz, 3H), 1.31 and 1.54 (2s, 6H), 4.25 (m, 1H), 4.77 (m, 1H), 5.32 (m, 1H), 6.28 (d, $J$ = 2.8 Hz, 1H), 8.22 (s, 1H) and 8.72 (s, 1H). Anal. ($C_{14}H_{15}N_3O_3ClI$) C, H, N, Cl, I.

**4-Chloro-5-bromo-7-(5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9c).** Glycosylation of **3b** with **8b** as described for **9a** gave **9c** as a glassy solid in 40% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.29 (d, $J$ = 6.3 Hz, 3H), 1.33 and 1.63 (2s, 6H), 4.3 (m, 1H), 4.55 (m, 1H), 5.25 (m, 1H), 6.22 (d, $J$ = 3.0 Hz, 1H), 8.28 (s, 1H) and 8.72 (s, 1H). Anal. ($C_{14}H_{15}N_3O_3ClBr$) C, H, N, Cl, Br.

**4-Chloro-5-methyl-7-(5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9d).** Glycosylation of **4a**[28] with **8b** as described for **9a** gave **9d** as a glassy solid in 38% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.22 (d, $J$ = 6.5 Hz, 3H), 1.30 and 1.55 (2s, 6H), 2.42 (s, 3H), 4.17 (m, 1H), 4.83 (m, 1H), 5.27 (m, 1H), 6.25 (d, $J$ = 3.75 Hz, 1H), 7.69 (s, 1H), 8.62 (s, 1H).

**4-Chloro-5-methylthio-7-(5-deoxy-2,3-*O*-isopropylidene*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9e).** Glycosylation of **4b** with **8b** as described for **9a** gave **9e** as a glassy solid in 29% yield: $^1$H NMR ($CDCl_3$) $\delta$ 1.31 (d, $J$ = 6.4 Hz, 3H), 1.25 and 1.62 (2s, 6H), 2.5 (s, 3H), 4.32 (m, 1H), 4.68 (m, 1H), 5.25 (m, 1H), 6.19 (d, $J$ = 3.5 Hz, 1H), 7.3 (s, 1H), 8.65 (s, 1H).

**4-Chloro-5-ethoxycarbonyl-7-(5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9f).** Glycosylation of **4c** with **8b** as described for **9a** gave **9f** as a glassy solid in 26% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.45 (m, 12H), 4.35 (q, 2H), 6.21 (d, $J$ = 3.0 Hz, 1H), 7.28 (s, 1H), 8.08 (s, 1H).

**4-Chloro-5-iodo-7-(5-azido-5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9g).** Glycosylation of **3a** with **8c** as described for **9a** gave **9g** as a glassy solid in 28% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 and 1.55 (2s, 6H), 3.53 (m, 2H), 4.29 (m, 1H), 4.92 (m, 1H), 5.36 (m, 1H), 6.29 (d, $J$ = 2.8 Hz, 1H), 8.21 (s, 1H), 8.70 (s, 1H).

**4-Chloro-5-bromo-7-(5-azido-5-deoxy-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9h).** Glycosylation of **3b** with **8c** as described for **9a** gave **9h** as a glassy solid in 25% yield: $^1$H NMR ($CDCl_3$) $\delta$ 1.40 and 1.65 (2s, 6H), 3.65 (m, 2H), 4.37 (m, 1H), 4. 95 (m, 1H), 5.23 (m, 1H), 6.31 (d, $J$ = 2.9 Hz, 1H), 7.49 (s, 1H), 8.29 (s, 1H); IR (KBr) 2210 cm$^{-1}$.

**4-Chloro-5-iodo-7-(5-*O*-methyl-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9i).** The chloro sugar **8d** was prepared from **7c** as described for **8b** and used immediately in the glycosylation of **3a** as described for **9a** to give **9i** as a glassy solid in 32% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.25 and 1.50 (2s, 6H), 3.33 (s, 3H) 3.55 (m, 2H), 4.35 (m, 1H), 4.83 (m, 1H), 5.28 (m, 1H), 6.32 (d, $J$ = 2.95 Hz, 1H), 8.11 (s, 1H), 8.7 (s, 1H).

**4-Chloro-5-iodo-7-(5,6-didehydro-5,6-dideoxy-2,3-*O*-isopropylidene-*β*-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9j).** The chloro sugar **8e** was prepared from **7d** as described for **8b** and used immediately in the glycosylation of **3a** as described for **9a** to give **9j** as a glassy solid in 45% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.35 and 1.62 (2s, 6H), 4.56 (m, 1H), 5.10 (m, 1H), 5.19 (m, 2H), 5.36 (m, 1H), 5.93 (m, 1H), 6.38 (d, $J$ = 3.3 Hz, 1H), 8.17 (s, 1H), 8.72 (s, 1H).

**4-Chloro-5-iodo-7-(5,6-dideoxy-2,3-*O*-isopropylidene-*β*-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9k).** The chloro sugar **8f** was prepared from **7e** as described for **8b** and used immediately in the glycosylation of **3a** as described for **9a** to give **9k** as a glassy solid in 48% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 0.86 (t, $J$ = 5.5 Hz, 3H), 1.33 and 1.55 (2s, 6H), 1.62 (m, 2H), 4.10 (m, 1H), 4.85 (m, 1H), 5.37 (m, 1H), 6.28 (d, $J$ = 2.6 Hz, 1H), 8.19 (s, 1H), 8.72 (s, 1H).

**4-Chloro-5-bromo-7-(5,6-dideoxy-2,3-*O*-isopropylidene-*β*-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (9l).** Glycosylation of **3b** with **8f** as described for **9a** gave **9l** as a glassy solid in 45% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 0.88 (t, $J$ = 5.5 Hz, 3H), 1.31 and 1.55 (2s, 6H), 1.62 (m, 2H), 3.98 (m, 1H), 4.77 (m, 1H), 5.30 (m, 1H), 6.30 (d, $J$ = 3.0, 1H), 8.23 (s, 1H), 8.75 (s, 1H).

**4-Chloro-5-iodo-7-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (10a).** A mixture of **9a** (500 mg, 0.9 mmol) and 70% TFA (30 mL) was stirred for 20 min at room temperature and concentrated under high vacuum. The residue was evaporated with water (20 mL) and the resulting solid was stirred with aqueous $NaHCO_3$ for 10 min. The product was collected by filtration, washed with water and crystallized from ethanol to give **10a** as microcrystals (222 mg, 60%): mp 190−193 °C (lit.[21] mp 194−196 °C); $^1$H NMR (DMSO-$d_6$) $\delta$ 3.62 (m, 2H), 3.95 (m, 1H), 4.13 (m, 1H), 4.45 (m, 1H), 5.13 (t, 1H, exchangeable with $D_2O$), 5.22 (d, $J$ = 5.4 Hz, 1H, exchangeable with $D_2O$), 5.44 (d, $J$ = 5.2 Hz,1H, exchangeable with $D_2O$), 6.21 (d, $J$ = 5.9 Hz, 1H), 8.26 (s, 1H), 8.69 (s, 1H).

**4-Chloro-5-iodo-7-(5-deoxy*β*-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (10b).** Deprotection of **9b** as described for **10a** gave **10b** as fine needles in 65% yield: mp 180−181 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.32 (d, $J$ = 6.6 Hz, 3H), 3.97 (m, 2H), 4.55 (m, 1H), 5.23 (d, $J$ = 6.0 Hz, 1H, exchangeable with $D_2O$), 5.45 (d, $J$ = 5.7 Hz, 1H, exchangeable with $D_2O$), 6.13 (d, $J$ = 5.8 Hz, 1H), 8.20 (s, 1H,), 8.69 (s, 1H). Anal. ($C_{11}H_{11}ClIN_3O_3$) C, H, Cl, N.

**4-Chloro-5-bromo-7-(5-deoxy-*β*-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (10c).** Deprotection of **9c** as described for **10a** gave **10c** as fine needles in 65% yield: mp 176−178 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.33 (d, $J$ = 6.5 Hz, 3H), 3.85−405 (m, 2H), 4.50 (m, 1H), 5.22 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.45 (d, $J$ = 5.5 Hz, 1H, exchangeable with $D_2O$), 6.15 (d, $J$ = 5.5 Hz, 1H), 8.22 (s, 1H), 8.72 (s, 1H). Anal. ($C_{11}H_{11}ClBrN_3O_3$) C, H, Cl, N.

**4-Chloro-5-methyl-7-(5-deoxy-*β*-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (10d).** Deprotection of **9d** as described for **10a** gave **10d** as microplates in 63% yield: mp 155−156 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.28 (d, $J$ = 6.2 Hz, 3H), 2.42 (s, 3H), 3.95 (m, 2H), 4.32 (m, 1H), 5.05 (br s, $J$ = 6.2 Hz, 1H, exchangeable with $D_2O$), 5.55 (br s, 1H, exchangeable with $D_2O$), 6.15 (d, $J$ = 5.78 Hz, 1H), 7.68 (s, 1H), 8.58 (s, 1H). Anal. ($C_{12}H_{14}ClN_3O_3$) C, H, N.

**4-Chloro-5-methylthio-7-(5-deoxy-*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10e).** Deprotection of **9e** as described for **10a** gave **10e** as fine needles in 62% yield: mp 147−148 °C;$^1$H NMR (DMSO-$d_6$) $\delta$ 1.33 (d, $J$ = 6.1 Hz, 3H), 2.45 (s, 3H), 3.93 (m, 2H), 4.51 (m, 1H), 5.20 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.42 (d, $J$ = 5.8 Hz, 1H, exchangeable with $D_2O$), 6.15 (d, $J$ = 5.4 Hz), 7.82 (s, 1H), 8.65 (s, 1H). Anal. ($C_{12}H_{14}ClN_3O_3S$) C, H, N, S.

**4-Chloro-5-iodo-7-(5-azido-5-deoxy-*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10g).** Deprotection of **9g** as described for **10a** gave **10g** as soft needles in 60% yield: mp 171−172 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.55 (m, 2H), 4.15 (m, 2H), 4.56 (m, 1H), 5.28 (br s, 1H, exchangeable with $D_2O$), 5.65 (br s, 1H, exchangeable with $D_2O$), 6.19 (d, $J$ = 5.92 Hz, 1H), 8.10 (s, 1H), 8.68 (s, 1H). Anal. ($C_{11}H_{10}ClIN_6O_3\cdot H_2O$) C, H, Cl, I, N.

**4-Chloro-5-bromo-7-(5-azido-5-deoxy-*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10h).** Deprotection of **9h** as described for **10a** gave **10h** as soft needles in 58% yield: mp 156−158 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.65 (m, 2H), 4.07 (m, 2H), 4.57 (m, 1H), 5.30 (d, $J$ = 5.6 Hz, 1H, exchangeable with $D_2O$), 5.65 (d, $J$ = 5.2 Hz, 1H, exchangeable with $D_2O$), 6.25 (d, $J$ = 5.9 Hz, 1H), 8.28 (s, 1H), 8.74 (s, 1H). Anal. ($C_{11}H_{10}BrClN_6O_3$) C, H, Br, Cl, N.

**4-Chloro-5-iodo-7-(5-*O*-methyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (10i).** Deprotection of **9i** as described for **10a** gave **10i** as microplates in 50% yield: mp 179180 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.28 (s, 3H), 3.55 (m, 2H), 4.05 (m, 2H),

4.45 (m, 1H), 5.25 (d, $J$ = 5.5 Hz, 1H, exchangeable with $D_2O$), 5.55 (d, $J$ = 5.2 Hz, 1H, exchangeable with $D_2O$), 6.19 (d, $J$ = 5.9 Hz, 1H), 8.12 (s, 1H), 8.68 (s, 1H). Anal. ($C_{12}H_{13}ClIN_3O_4$) C, H, N.

**4-Chloro-5-iodo-7-(5,6-didehydro-5,6-dideoxy-β-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (10j).** Deprotection of **9j** as described for **10a** gave **10j** as fine needles in 75% yield: mp 217−219 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.95−4.61 (m, 3H), 5.15−5.46 (m, 4H,), 6.10 (m, 1H), 6.23 (d, $J$ = 5.75 Hz, 1H), 8.21 (s, 1H), 8.70 (s, 1H).

**4-Chloro-5-iodo-7-(5,6-dideoxy-β-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (10k).** Deprotection of **9k** as described for **10a** gave **10k** as microplates in 75% yield: mp 181−183 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.95 (t, $J$ = 5.5 Hz, 3H), 1.70 (m, 2H), 3.78 (m, 1H), 3.95 (m, 1H), 4.48 (m, 1H), 5.15 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.46 (d, $J$ = 5.6 Hz, 1H, exchangeable with $D_2O$), 6.17 (d, $J$ = 5.85 Hz, 1H), 8.18 (s, 1H), 8.69 (s, 1H).

**4-Chloro-5-bromo-7-(5,6-dideoxy-β-D-allofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10l).** Deprotection of **9l** as described for **10a**, gave **10l** as microcrystals in 80% yield: mp 163−165 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.92 (t, $J$ = 5.6 Hz, 3H), 1.67 (m, 2H), 3.72−4.6 (m, 3H), 5.18 (d, $J$ = 6.1 Hz, 1H, exchangeable with $D_2O$), 5.50 (d, $J$ = 5.8 Hz, 1H, exchangeable with $D_2O$), 6.21 (d, $J$ = 5.65 Hz, 1H), 8.23 (s, 1H), 8.73 (s, 1H).

**4-Amino-5-iodo-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine (1a).** A mixture of **10a** (1.0 g, 2.4 mmol) and saturated methanolic ammonia (40 mL) was heated in a steel bomb for 16 h in a 100−120 °C oil bath. The bomb was cooled, opened, and the unreacted $NH_3$ was allowed to evaporate. Concentration of the reaction mixture gave a crude product, which was crystallized from ethanol to give **1a** (0.7 g, 75%): mp 217−219 °C (lit.[21] mp 217 °C).

**4-Amino-5-iodo-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (1b).** Amination of **10b** as described for **1a** gave **1b** as needles from aqueous ethanol: yield 65%; mp 232−233 °C (lit.[23] mp 234−235 °C).

**4-Amino-5-bromo-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (1c).** Amination of **10c** as described for **1a** gave **1c** as needles from aqueous ethanol in 60% yield: mp 244−246 °C (lit.[23] mp 245−247 °C).

**4-Amino-5-methyl-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (11a).** Amination of **10d** as described for **1a** gave **11a** as soft needles from aqueous ethanol in 65% yield: mp 212−214 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.22 (d, $J$ = 6.5 Hz, 3H), 2.31 (s, 3H), 3.87 and 4.33 (2m, 3H), 5.05 (d, $J$ = 5.4 Hz,1H, exchangeable with $D_2O$), 5.28 (d, $J$ = 5.3 Hz, 1H, exchangeable with $D_2O$), 5.98 (d, $J$ = 5.2 Hz, 1H), 6.65 (m, 2H, exchangeable with $D_2O$), 7.05 (s, 1H), 8.05 (s, 1H). Anal. ($C_{12}H_{16}N_4O_3$) C, H, N.

**4-Amino-5-methylthio-7-(5-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11b).** Amination of **10e** as described for **1a** gave **11b** as pale yellow needles from aqueous ethanol in 60% yield: mp 217−218 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.29 (d, $J$ = 6.2 Hz, 3H), 2.41 (s, 3H), 3.80−4.05 (m, 2H), 4.45 (t, 1H), 4.70 (br s, 1H, exchangeable with $D_2O$), 5.55 (br s, 1H, exchangeable with $D_2O$), 6.02 (d, $J$ = 5.3 Hz, 1H), 7.70 (s, 1H), 8.0 (br s, 2H, exchangeable with $D_2O$), 8.32 (s, 1H). Anal. ($C_{12}H_{16}N_4O_3S\cdot CF_3COOH$) C, H, N, S.

**4-Amino-5-ethoxycarbonyl-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11c).** A solution of **9f** (580 mg 1.5 mmol) was subjected to amination as described for **1a** and the resulting crude material was subjected to TFA catalyzed deprotection to give **11c** as microplates from boiling ethanol (0.268 g, 55% overall from **9f**): mp 166−168 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.35 (m, 6H), 3.92 and 4.55 (2m, 3H), 4.35 (m, 2H), 5.05 (br s, 1H, exchangeable with $D_2O$), 5.28 (br s, 1H, exchangeable with $D_2O$), 6.06 (d, $J$ = 5.87 Hz, 1H), 7.55 (br s, 1H, exchangeable with $D_2O$), 7.90 (br s, 1H, exchangeable with $D_2O$), 8.15 (s, 1H), 8.20 (s, 1H). Anal. ($C_{14}H_{18}N_4O_5$) C, H, N.

**4-Amino-5-iodo-7-(5-azido-5-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11d).** Amination of **10g** as described for **1a** gave **11d** as fine needles from aqueous ethanol in 58% yield: mp 176−178 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.25 (m, 2H), 3.8 and 4.22 (2m, 3H), 5.10 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.26 (d, $J$ = 5.7 Hz, 1H, exchangeable with $D_2O$), 5.85 (d, $J$ = 6.02 Hz, 1H), 6.55 (br s, 2H, exchangeable with $D_2O$), 7.45 (s, 1H), 7.90 (s, 1H); IR (KBr) 2240 cm$^{-1}$. Anal. ($C_{11}H_{12}IN_7O_3$) C, H, N.

**4-Amino-5-bromo-7-(5-azido-5-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11e).** Amination of **10h** as described for **1a** gave **11e** as soft needles from aqueous ethanol in 58% yield: mp 182−183 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.57 (m, 2H), 3.85 and 4.25 (2m, 2H), 4.42 (m, 1H), 5.34 (d, $J$ = 6.2 Hz, 1H, exchangeable with $D_2O$), 5.50 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 6.09 (d, $J$ = 5.75 Hz, 1H), 6.75 (br s, 2H, exchangeable with $D_2O$), 7.64 (s, 1H), 8.12 (s, 1H). Anal. ($C_{11}H_{12}BrN_7O_3$) C, H, N.

**4-Amino-5-iodo-7-(5-*O*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11f).** Amination of **10i** as described for **1a** gave **11f** which was crystallized from aqueous ethanol as microplates in 62% yield: mp 216−218 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.33 (s, 3H), 3.55 (m, 2H), 4.05 (m, 2H), 4.45 (m, 1H), 5.25 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.55 (d, $J$ = 5.6 Hz, 1H, exchangeable with $D_2O$), 6.17 (d, $J$ = 5.58 Hz, 1H), 6.80−7.20 (br s, 2H, exchangeable with $D_2O$), 8.15 (s, 1H), 8.69 (s, 1H). Anal. ($C_{12}H_{15}IN_4O_4$) C, H, N.

**4-Amino-5-iodo-7-(5,6-didehydro-5,6-dideoxy-β-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11g).** Amination of **10j** as described for **1a** gave **11g** as needles from aqueous ethanol in 68% yield: mp 224−225 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.95−4.5 (m, 3H), 5.22 (m, 2H), 5.35 (d, $J$ = 5.4 Hz, 1H, exchangeable with $D_2O$), 5.55 (d, $J$ = 5.2 Hz, 1H, exchangeable with $D_2O$), 6.02 (m, 1H), 6.10 (d, $J$ = 5.95 Hz, 1H), 6.75 (br s, 2H, exchangeable with $D_2O$), 7.62 (s, 1H), 8.13 (s, 1H). Anal. ($C_{12}H_{13}IN_4O_3$) C, H, N.

**4-Amino-5-iodo-7-(5,6-dideoxy-β-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11h).** Amination of **10k** as described for **1a** gave **11h** as needles from ethanol in 70% yield: mp 233−234 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.92 (t, $J$ = 5.5 Hz, 3H), 1.64 (m, 2H), 3.72 and 3.93 (2m, 2H), 4.43 (m, 1H), 5.05 (d, $J$ = 5.5 Hz, 1H, exchangeable with $D_2O$), 5.38 (d, $J$ = 5.3 Hz, 1H, exchangeable with $D_2O$), 6.02 (d, $J$ = 5.65 Hz, 1H), 6.75 (br s, 2H, exchangeable with $D_2O$), 7.65 (s, 1H), 8.15 (s, 1H). Anal. ($C_{12}H_{15}IN_4O_3$) C, H, N.

**4-Amino-5-bromo-7-(5,6-dideoxy-β-D-allofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11i).** Amination of **10l** as described for **1a** gave **11i** as needles from aqueous ethanol in 58% yield: mp 229−230 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.95 (t, $J$ = 5.8 Hz, 3H), 1.62 (m, 2H), 3.65−4.45 (m, 3H), 5.05 (d, $J$ = 5.6 Hz, 1H, exchangeable with $D_2O$), 5.33 (d, $J$ = 5.4 Hz, 1H, exchangeable with $D_2O$), 6.05 (d, $J$ = 5.81 Hz, 1H), 6.80 (br s, 2H, exchangeable with $D_2O$), 7.60 (s, 1H), 8.12 (s, 1H). Anal. ($C_{12}H_{15}BrN_4O_3$) C, H, N.

**4-*N*-Methylamino-5-iodo-7-(5-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11j).** Amination of **10b** with aqueous methylamine solution as described for **1a** gave **11j**, which was chromatographed on silica gel (10% methanol in methylene chloride), and the product was crystallized from aqueous ethanol to give **11j** as needles in 58% yield: mp 226−227 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.25 (d, $J$ = 6.4 Hz, 3H), 3.05 (d, $J$ = 7.0 Hz, 3H), 3.85 (m, 2H), 4.37 (m, 1H), 5.10 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.35 (d, $J$ = 5.5 Hz, 1H, exchangeable with $D_2O$), 6.01 (d, $J$ = 5.7 Hz, 1H), 6.44 (m, 1H, exchangeable with $D_2O$), 7.60 (s, 1H), 8.21 (s, 1H). Anal. ($C_{12}H_{15}IN_4O_3$) C, H, N.

**4-Chloro-5-iodo-7-(5-azido-5-deoxy-2,3-*O*-isopropylidene-α-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (12).** A solution of **8c** (prepared from 1.3 g of **7b** as described for **8b**) was warmed to 55 °C over a period of 2 h and added to a stirred solution of **3a** (0.87 g, 3.1 mmol) and NaH (103 mg of 80% in oil, 3.4 mmol) in dry acetonitrile. The reaction mixture was stirred at room temperature for 16 h and worked up as described for **9a** to give **12** as a glassy solid (0.43 g, 32%): $^1$H NMR (DMSO-$d_6$) $\delta$ 1.15 and 1.25 (2s, 6H), 3.62 (m, 2H), 4.55 (m, 1H), 4.76 (d, 1H), 4.92 (t, 1H), 6.70 (d, $J$ = 4.5 Hz, 1H), 7.80 (s, 1H), 8.61 (s, 1H); IR (KBr) 2210 cm$^{-1}$.

**4-Chloro-5-iodo-7-(5-azido-5-deoxyα-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (13).** Compound **12** was subjected to deprotection as described for **10a** using 70% TFA to give **13** as microplates from ethanol in 70% yield: mp 202−204 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.55 (m, 2H), 4.10 (m, 1H), 4.45 (m, 2H), 5.39 (br s, 1H, exchangeable with $D_2O$), 5.55 (br s, 1H, exchangeable with $D_2O$), 6.65 (d, $J$ = 7.92 Hz, 1H), 8.10 (s, 1H), 8.65 (s, 1H); IR (KBr) 2207 cm$^{-1}$.

**5-Iodo-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-thione (14a).** A mixture of **10b** (3.95 g, 10 mmol), thiourea (1.5 g, 20 mmol) and ethanol (50 mL) was heated to reflux overnight and the solvent removed under reduced pressure. The residue was stirred with cold ethanol (2 × 10 mL) and the resulting solid crystallized from boiling ethanol (3.25 g, 80%): mp 161−163 °C; UV (methanol and pH 7): $\lambda_{max}$ 234 ($\epsilon$ 14900), 334 ($\epsilon$ 16900), 284 nm ($\epsilon$ 9090); (pH 9) $\lambda_{max}$ 234 ($\epsilon$ 16700), 326 nm ($\epsilon$ 14200); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 (d, $J$ = 6.1 Hz, 3H), 3.83 (m, 1H), 4.45 (m, 2H), 5.13 (d, $J$ = 6.1 Hz, 1H, exchangeable with $D_2O$), 5.4 (d, $J$ = 6.0 Hz, 1H, exchangeable with $D_2O$), 5.98 (d, $J$ = 5.38 Hz, 1H), 7.71 (s, 1H) and 8.07 (s, 1H). Anal. ($C_{11}H_{12}IN_3O_3S$) C, H, N, S.

**5-Iodo-4-methylthio-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (14b).** To a stirred suspension of **14a** (786 mg, 2 mmol) in water (10 mL) was added concentrated $NH_4OH$ solution until a clear solution formed. Iodomethane (0.355 g, 2.5 mmol) was added to the reaction mixture and stirred at room-temperature overnight. The resulting precipitate was collected by filtration, washed with water and crystallized from boiling ethanol to give **14b** as needles from ethanol in 70% yield: mp 215−216 °C; $^1$H NMR (DMSO-$d_6$) 1.28 (d, $J$ = 6.3 Hz, 3H), 2.64 (s, 3H), 3.80 (m, 1H), 4.65 (m, 2H), 5.16 (d, $J$ = 5.8 Hz, 1H, exchangeable with $D_2O$), 5.40 (d, $J$ = 5.5 Hz, 1H, exchangeable with $D_2O$), 6.10 (d, $J$ = 5.15 Hz, 1H), 7.91 (s, 1H) and 8.61 (s, 1H). Anal. ($C_{12}H_{14}IN_3O_3S\cdot H_2O$) C, H, N, S.

**5-Iodo-4-allylthio-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (14c).** Alkylation of **14a** using allyl iodide as described for **14b** gave **14c** as pale yellow needles from ethanol in 55% yield: mp 192−193 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.25 (d, $J$ = 6.5 Hz, 3H), 3.88 (m, 1H), 4.43 (m, 2H), 4.05 (s, 2H), 5.16 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.45 (d, $J$ = 5.8 Hz, 1H, exchangeable with $D_2O$), 5.10 (d, 1H), 5.35 (d, 1H), 5.9 (m, 1H), 6.12 (d, $J$ = 5.45 Hz, 1H), 7.95 (s, 1H) and 8.62 (s, 1H). Anal. ($C_{14}H_{16}IN_3O_3S$) C, H, N, S.

**5-Iodo-4-*n*-butylthio-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (14d).** Alkylation of **14a** using *n*-butyl iodide as described for **14b** gave **14d** as needles from ethanol in 65% yield: mp 186−187 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.95 (t, 3H), 1.30 (d, $J$ = 6.6 Hz, 3H), 1.35 (m, 2H) 1.8 (m, 2H), 3.30 (t, 2H), 3.8 (m, 2H), 4.5 (m, 1H), 5.15 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.38 (d, $J$ = 5.7 Hz, 1H, exchangeable with $D_2O$), 6.13 (d, $J$ = 5.25 Hz, 1H), 7.94 (s, 1H) and 8.60 (s, 1H). Anal. ($C_{15}H_{20}IN_3O_3S$) C, H, N, S.

**5-Iodo-4-benzylthio-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (14e).** Alkylation of **14a** using benzyl bromide as described for **14b** gave **14e**, which was crystallized from ethanol as microcrystals in 75% yield: mp 224−226 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.29 (d, $J$ = 6.3 Hz, 3H), 3.9 (m, 2H), 4.45 (m, 1H), 4.62 (s, 2H), 5.18 (d, $J$ = 5.7 Hz, 1H, exchangeable with $D_2O$), 5.40 (d, $J$ = 5.6 Hz, 1H, exchangeable with $D_2O$), 6.10 (d, $J$ = 5.36 Hz, 1H), 7.20−7.50 (2m, 5H), 7.92 (s, 1H) and 8.68 (s, 1H). Anal. ($C_{18}H_{18}IN_3O_3S$) C, H, N, S.

**5-Iodo-4-(4-nitrobenzyl)thio-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (14f).** Alkylation of **14a** using *p*-nitrobenzyl bromide by the as described for **14b** gave **14f**, which was crystallized from ethanol as pale yellow plates in 72% yield: mp 218−220 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.26 (d, $J$ = 6.2 Hz, 3H), 3.9 (m, 1H) 4.45 (m, 2H), 4.78 (s, 2H, $CH_2$), 5.15 (d, $J$ = 5.9 Hz, 1H, exchangeable with $D_2O$), 5.38 (d, $J$ = 5.6 Hz, 1H, exchangeable with $D_2O$), 6.10 (d, $J$ = 5.25 Hz, 1H), 7.77 (d, 2H), 8.2 (d, 2H), 7.94 (s, 1H) and 8.65 (s, 1H). Anal. ($C_{18}H_{17}IN_4O_5S$) C, H, N, S.

**4-Amino-5-iodo-7-(5-amino-5-deoxyβ-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (15a).** The intermediate **9g** was subjected to amination as described for **1a** to give 4-amino-5-iodo-7-(5-azido-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine as a glassy solid in 64% yield: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 and 1.50 (2s, 6H), 3.57 (d, 2H), 4.20 (m, 1H), 4.85 (m, 1H), 5.30 (m, 1H), 6.21 (d, $J$ = 3.7 Hz, 1H), 6.75 (br s, 2H, exchangeable with $D_2O$), 7.69 (s, 1H), 8.15 (s, 1H). A solution of this intermediate (1.98 g, 4.33 mmol) in dry THF (25 mL) was treated with triphenylphosphine (1.59 g, 6.06 mmol) and stirred overnight. The reaction mixture was then treated with concentrated $NH_4OH$ solution (3 mL) and the resulting solution refluxed for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue chromatographed on silica gel (20% methanol in methylene chloride) to give 4-amino-5-iodo-7-(5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine as a foam (1.7 g, 91%): $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30 and 1.52 (2s, 6H), 2.35 (br s, 2H, exchangeable with $D_2O$), 3.72 (m, 2H), 4.0 (m, 1H), 4.85 (m, 1H), 5.25 (m, 1H), 6.15 (d, $J$ = 3.7 Hz, 1H), 6.75 (br s, 2H, exchangeable with $D_2O$), 7.71 (s, 1H), 8.12 (s, 1H). This compound was deprotected with 70% TFA as described for **10a**. The resulting product was dissolved in water (50 mL) and filtered through a Celite pad. The pH of the filtrate was adjusted to ∼8 with 3 N NaOH solution and allowed to stand in the refrigerator overnight. The resulting precipitate was collected by filtration, washed with water and dried under vacuum to give **15a** as a white solid (1.22 g, 81%): mp 205−208 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.65 (br s, 2H, exchangeable with $D_2O$), 2.75 (m, 2H), 4.10 (m, 3H), 4.95 (m, 1H), 5.45 (m, 2H, exchangeable with $D_2O$), 6.01 (d, $J$ = 5.75 Hz, 1H), 6.75 (br s, 2H, exchangeable with $D_2O$), 7.69 (s, 1H) and 8.10 (s, 1H). Anal. ($C_{11}H_{14}IN_5O_3$) C, H, I, N.

**4-Amino-5-bromo-7-(5-amino-5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (15b).** Compound **9h** (0.78 g, 1.8 mmol) was first subjected to amination as described for **1a** to give 4-amino-5-bromo-7-(5-azido-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine as a glassy material (0.59 g, 79%): $^1$H NMR ((DMSO-$d_6$) $\delta$ 1.30 and 1.52 (2s, 6H), 3.57 (d, 2H), 4.25 (m, 1H), 4.85 (m, 1H), 4.95 (m, 1H), 6.21 (d, $J$ = 5.12 Hz, 1H), 6.95 (br s, 2H, exchangeable with $D_2O$), 7.65 (s, 1H), 8.12 (s, 1H). This intermediate (560 mg, 1.36 mmol) and triphenylphosphine (500 mg, 1.9 mmol) were dissolved in anhydrous THF (25 mL), and worked up as described in the case of **15a** to give 4-amino-5-bromo-7-(5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine as a dark brown solid (0.49 g, 93%): $^1$H NMR (DMSO-$d_6$) $\delta$ 1.25 and 1.5 (2s, 6H), 1.85 (br s, 2H, exchangeable with $D_2O$), 2.72 (d, 2H), 4.05 (m, 1H), 4.90 (m, 1h), 5.17 (m, 1H), 6.15 (d, $J$ = 5.1 Hz, 1H), 6.95 (br s, 2H, exchangeable with $D_2O$), 7.70 (s, 1H), 8.10 (s, 1H). This material was dissolved in 70% TFA, stirred at room temperature for 45 min and then evaporated to dryness under reduced pressure. The residue was coevaporated with water (2 × 20 mL) to remove excess acid and then redissolved in water (15 mL) before being applied to a short column of Dowex-50W, $H^+$ until all the product was absorbed onto the resin. The column was eluted with water (6 × 50 mL) and the effluent was discarded. Further elution of the column with 1 M $NH_4OH$ in MeOH and water (60:40) gave UV absorbing fractions, which were pooled and evaporated to furnish a semisolid. Repeated evaporation of this product with ethanol gave pure **15b** (0.22 g, 51%) as an amorphous powder with no sharp melting point: $^1$H NMR (DMSO-$d_6$) $\delta$ 2.95 (m, 2H), 3.45 (br s, 1H, exchangeable with $D_2O$) 4.20 (m, 3H), 5.45 (br s, 2H, exchangeable with $D_2O$), 6.05 (d, $J$ = 6 Hz, 1H), 6.85 (br s, 2H, exchangeable with $D_2O$), 7.80 (s, 1H), and 8.12 (s, 1H). Anal. ($C_{11}H_{14}BrN_5O_3\cdot H_2O$) C, H, Br, N.

**4-Chloro-5-iodo-7-(5-amino-5-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (16).** To a solution of **9g** (1.05 g, 2.2 mmol) in anhydrous THF (40 mL) was added triphenylphosphine (0.8 g, 3 mmol). This mixture stirred at room temperature for 4 h. The resulting solution was then treated with concentrated $NH_4OH$ (8 mL) and stirred for 2 h. The reaction mixture was worked up as in case of **15a** to give 530 mg of 4-chloro-5-iodo-7-(5-amino-5-deoxy-2,3-*O*-isopropylidene-

β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine as a crude product. This crude product was dissolved in 1 N HCl (10 mL) and stirred for 3 h to complete the deprotection of the isopropylidene group. The reaction mixture was diluted with ethanol (10 mL) and evaporated under reduced pressure. The residue was evaporated with ethanol to give a solid, which was dissolved in 1 N HCl (2 mL) and allowed to stand at room temperature for 2 days. The solution was diluted with ethanol (5 mL) and refrigerated for 3 h. The resulting white precipitate was collected by filtration, washed with ethanol and dried in vacuum to give **16** as a HCl salt (175 mg, 18%). This product did not show a sharp melting point: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.25 (m, 2H), 4.05−4.20 (m, 2H), 4.55 (m, 1H), 5.45 (d, $J$ = 6.3 Hz, 1H, exchangeable with $D_2O$), 5.70 (d, $J$ = 6.1 Hz, 1H, exchangeable with $D_2O$), 6.21 (d, $J$ = 5.9 Hz, 1H), 8.05 (br s, 3H, exchangeable with $D_2O$), 8.30 (s, 1H), 8.70 (s, 1H). Anal. ($C_{11}H_{12}ClIN_4O_3$·HCl) C, H, N, Cl.

**Enzyme Assay.** AK activity was measured in a radiochemical assay similar to the procedure of Yamada et al.,[34] with minor modifications. The final reaction volume was 100 μL and contained 70 mM Tris-maleate (pH 7.0), 0.1% (w/v) bovine serum albumin, 1.0 mM $MgCl_2$, 1.0 mM ATP, 1.0 μM [U-$^{14}$C]adenosine (400−600 mCi/mmol; Moravek Biochemicals, Inc.) and various inhibitor concentrations. Inhibitors were prepared as 10 mM stock solutions in DMSO. The final DMSO concentration in the assay was 5% (v/v). Eleven different concentration of the test solutions ranging from 0.001 to 10.0 μM were utilized to determine a dose response curve of the inhibition of the enzyme. Reactions were started by adding the appropriate amount of purified human recombinant AK and incubated for 20 min at 37 °C. The reactions were terminated by addition of the potent AKI GP3269.[40] A 30-μL aliquot of each reaction was spotted on DEAE cellulose filter paper (cut in squares of ∼1 × 1 cm) and air-dried for 30 min. The dry filters were then washed for 3 min in deionized water to remove residual [U-$^{14}$C]adenosine, rinsed with ethanol and dried at 90 °C for 20 min. The filter papers were counted in 5.5 mL of Ready Safe liquid scintillation cocktail using a Beckman LS3801 scintillation counter. Control AK activity was determined from the amount of [$^{14}$C]AMP formed in the presence of 5% DMSO. The concentration of inhibitor required to inhibit 50% of the AK activity ($IC_{50}$) was determined graphically from plots of inhibitor concentration versus percent (%) control enzyme activity. The results are shown in Table 1.

**MES Seizure Assay.** Male SA rats (100−150 g; Simonsen) were maintained on a 12:12 light:dark cycle in temperature-controlled facilities with free access to food and water. One hour prior to seizure testing, the animals were injected intraperitoneally (1 mL/kg) with DMSO vehicle or with test compound dissolved in DMSO. At the time of the test, an electrolyte solution (2% lidocaine in 0.9% sodium chloride) was applied to the eyes. Maximal electroshock seizures were induced by administering a 60-Hz current of 150 mA for 0.2 s via corneal electrodes, using a Wahlquist Model H stimulator (Wahlquist Instrument Co.).[41] The endpoint measured was suppression of hindlimb tonic extension (HTE) and expressed as percentage of animals in which the response was inhibited. At this supramaximal stimulation level, virtually 100% of control (vehicle-treated) animals showed HTE. $ED_{50}$ values were calculated from a dose−response curve using probit analysis.[42] The $N$ for the screening doses was 6−8; dose−response determinations were conducted with at least 5 animals/dose.

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