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Article

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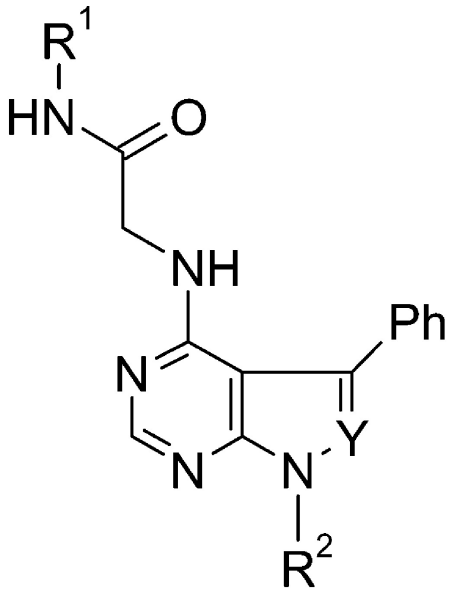

R^1 = alkyl, cycloalkyl, aryl, etc.
Y = CH, N
R^2 = 5'-deoxyribose, ribose,
erythrose and $CH_2O(CH_2)_2OH$
AK IC_{50}s < 10 nM

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4-(Phenylamino)-5-phenyl-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1**) and related compounds known as "diaryltubercidin" analogues are potent inhibitors of adenosine kinase (AK) and are orally active in animal models of pain such as the rat formalin paw model (GP3269 $ED_{50} = 6.4$ mg/kg). However, the utility of this compound class is limited by poor water solubility that can be attributed to the high energy of crystallization caused by stacking of the parallel C4 and C5 aryl rings in the solid state (compound **1** and GP3269 each with pH 7.4 solubility <0.05 μg/mL). To increase water solubility, the hydrophobic C4-phenylamino substituent was replaced with a more hydrophilic group, glycinamide. This modification resulted in improved water solubility while retaining AK inhibition potency. Analogues were studied where changes in the glycinamide moiety were combined with changes to the base and sugar. A lead compound, 4-*N*-(*N*-cyclopropylcarbamoylmethyl)amino-5-phenyl-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**16c**) ($IC_{50} = 3$ nM and water solubility $= 32 \pm 9$ μg/mL at pH 7.4), was further characterized in biological assays. Compound **16c** exhibited strong oral efficacy in the rat formalin paw model (ED_{50} of 2.5 mg/kg). In the most advanced assay, **16c** was found to inhibit bradykinin-induced licking in marmoset monkeys with an ED_{50} estimated at 0.9 mg/kg without producing evidence of side effects such as ataxia, sedation, and emesis at this dose. However, lethal toxicity in the rat formalin paw model occurred with high doses of **16c**, and further work on this series was discontinued.

Introduction

Adenosine kinase inhibitors (AKIs) induce profound adenosine-mediated biological activity without hemodynamic side effects in animal models of epilepsy,[1] stroke,[2] inflammation,[3] and acute myocardial infarction.[4] For example, the prototypic diaryltubercidin analogue **1** inhibited seizure activity with an ED_{50} value of 1.1 mg/kg ip in a theophylline-reversible fashion.[1b,1d] This supports the hypothesis that the pharmacological effect of AKIs is mediated by adenosine receptor activation and is consistent with elevation of endogenous adenosine due to inhibition of AK. AKIs block adenosine metabolism at all sites but have their largest effect on local adenosine levels at sites with net ATP breakdown caused by tissue injury, ischemia, seizures, etc. Furthermore, AKIs have antinociceptive properties,[5] and detailed studies have been reported on the investigation of AKIs for the treatment of pain.[6]

The analgesic effect is realized as a result of a chain of events that begin with localized increases in purine metabolism that occur in the central nervous system (CNS) as a response to signaling events triggered by the onset of pain. AKIs are proposed to act in a site- and event-specific manner by blocking adenosine phosphorylation with the result being delivery of increased levels of adenosine to the extracellular space. The increased levels of adenosine activate adenosine receptors in the CNS to produce the antinociceptive response. The development of specific adenosine receptor agonists as drugs has been limited because their delivery cannot be localized sufficiently to avoid receptor-mediated cardiovascular side effects such as hypotension and bradycardia.[7] AKIs do not induce cardiovascular side effects, which indicates that the increased adenosine concentration is focused in the CNS. Localization is facilitated by the short half-life of adenosine (<5 s) preventing distribution beyond the site of production. Thus AKIs have been proposed to act as site- and event-specific "adenosine-regulating agents (ARAs)".[1−8]

Preliminary studies indicated that diaryltubercidin AKIs exhibit analgesic effects in several models of pain, including the rat formalin paw model[9] as well as the rat hot-plate[10] and tail-flick[11] models. For example, GP3269[1b,c] has an ED_{50} value of 6.4 mg/kg in the oral rat formalin paw model.[6a] Further development of these compounds, however, was impeded by their poor solubility characteristics (<0.05 μg /mL at pH 7.4) at physiological pH. Poor water solubility may result in decreased oral absorption, even if the compound has a good permeation rate across the intestinal mucosa into the circulation. In their studies of known drugs and the

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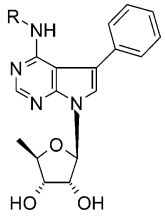
compounds recommended for development in man at Pfizer, Lipinski[12] and Curatolo[13] determined that for a targeted oral dose of 1 mg/kg, solubility should be at least 50 μg/mL. We sought to modify the structure of **1** to increase water solubility and thereby discover an AKI more suitable for development.



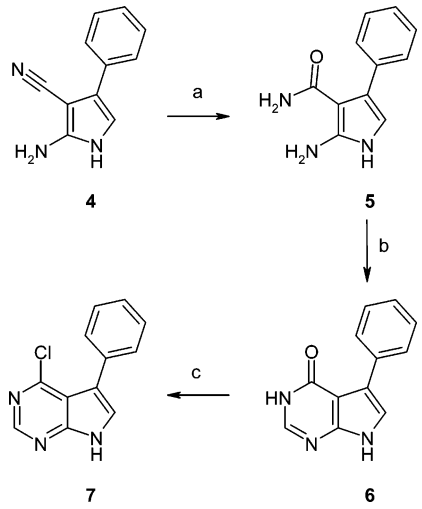
1, R = Ph
GP3269, R = *p*-F-Ph
2, R = H
3, R = cyclohexyl

The poor solubility of diaryltubercidins can be attributed to the high energy of crystallization from stacking of the C4 and C5 aryl rings in the solid state. Therefore, we hypothesized that replacement of one of the rings, specifically the C4-arylamino group, with a flexible side chain, especially one containing heteroatoms such as carbamoylalkyl, would attenuate the stacking effect and thereby increase the solubility properties of such molecules. However, at the onset of our studies it was not clear whether such a change would be tolerated by AK because previous inhibitor SAR indicated that replacement of a phenyl ring in the 4-position of **1** with H (**2**) or a cyclohexyl group (**3**) resulted in a loss of inhibitory potency (IC_{50}s = 0.32 μM and 1.2 μM, respectively).[1b] Additionally, while increasing solubility was considered important to enhancing oral bioavailability, it was uncertain whether this would compromise CNS penetration and thereby eliminate analgesic activity. To our surprise, a majority of the 4-[(carbamoylmethyl)amino] substituted (hereafter termed "glycinamide" substituted) compounds showed not only retention of AK inhibitory activity but also improved solubility in pH 7.4 buffered solutions. Furthermore, some of the compounds also exhibited significantly improved in vivo activity in a number of animal models of pain. This paper describes the synthesis, SAR, and solubility properties of 5-aryltubercidin analogues with a variety of C4 glycinamide substituents. The investigation resulted in the identification of a lead compound, **16c**, a pyrrolopyrimidine nucleoside with a C4 *N*-cyclopropylglycinamide moiety that met the necessary solubility, IC_{50}, and in vivo analgesic assay criteria to merit in depth analysis in advanced biological studies.

Chemistry

The synthesis of the pyrrolopyrimidine and pyrazolopyrimidine nucleosides described here required glycosylation reactions of appropriate bases and suitable sugar precursors. Pyrrolopyrimidine base **7** was prepared as shown in Scheme 1 from pyrrole **4**.[14] Hydrolysis of the nitrile produced amide **5**, which cyclized in the presence of triethyl orthoformate to form **6**. Phosphorus oxychloride-mediated chlorination resulted in **7** in 40% overall yield after three steps. For glycosylation of pyrrolopyrimidines, chloro sugars **9a**,[1a] **9b**,[1b] and **9c**[6a] were prepared in situ as described in Scheme 2 via reaction

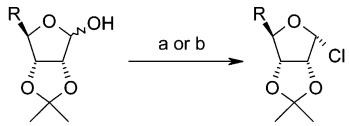
Scheme 1[a]



4 **5** **6** **7**

[a] (a) H_2SO_4, 100 °C (67%); (b) triethyl orthoformate, PTSA (72%); (c) $POCl_3$, 120 °C, 2.5 h (85%).

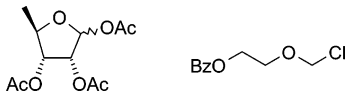
Scheme 2. Sugar and Sugar Mimic Glycosylation-Coupling Partners[a]



8a, R = CH_3
8b, R = CH_2OTBS
8c, R = H

9a, R = CH_3
9b, R = CH_2OTBS
9c, R = H

[a] (a) for **9a** and **9c** oxalylchloride, DMF, acetonitrile; (b) for **9b**: HMPT, CCl_4, toluene (see ref 1b).



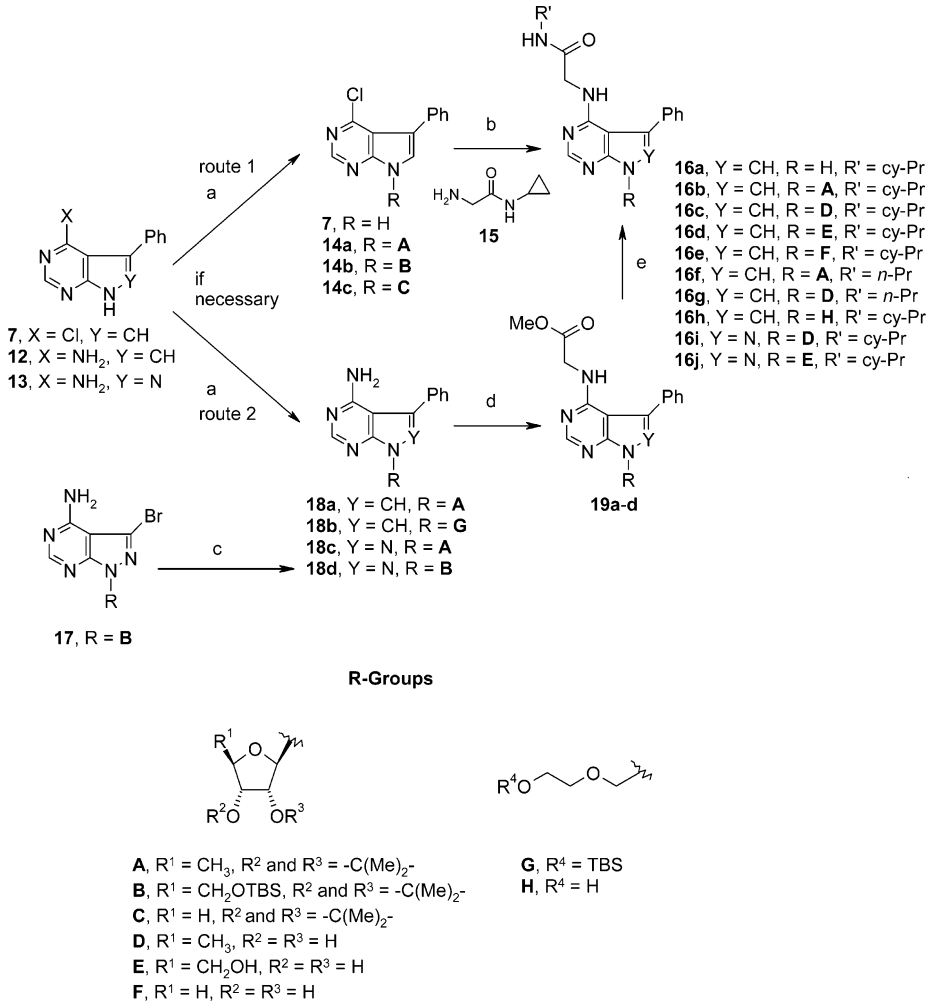
10 **11**

of the respective hydroxy sugars **8a**,[1a] **8b**,[15] and **8c**[16] with the Vilsmeyer salt[17] or HMPT/CCl_4.[1b] The use of the Vilsmeyer salt for anomeric chlorination[17] is a new, convenient method that proved very useful for the preparation of certain 1-chlororibosyl analogues.[6a] The triacetate **10** was prepared from **8a** by modification of a known method[18] and was used for the glycosylation of a pyrazolopyrimidine base.

The general methods used for the synthesis of glycinamide-substituted nucleosides are illustrated in Scheme 3 with the preparation of some representative examples.[19] The two approaches used required the preparation of 4-chloropyrrolopyrimidine nucleosides (route 1) or 4-aminopyrrolopyrimidine and 4-aminopyrazolopyrimidine nucleosides (route 2). The 4-chloropyrrolopyrimidine nucleosides **14a**−**c** were prepared from a tris(2-(2-methoxyethoxy)ethyl)amine (TDA-1)-mediated glycosylation reaction[1b] between the potassium salt of the base **7** and chloro sugars **9a**−**c**, respectively. The chlorine in compounds **7** and **14a**−**c** was displaced in high yield with amine nucleophiles (such as **15** in these examples), and the sugar (if present) was routinely deprotected with 70% TFA in water to provide the desired compounds **16a** and **16c**−**e**, respectively.

Route 2 of Scheme 3 required the preparation of 4-aminopyrrolopyrimidine and 4-aminopyrazolopyrimi-

Scheme 3. Synthesis of Representative Glycinamide-Substituted Nucleosides[a]

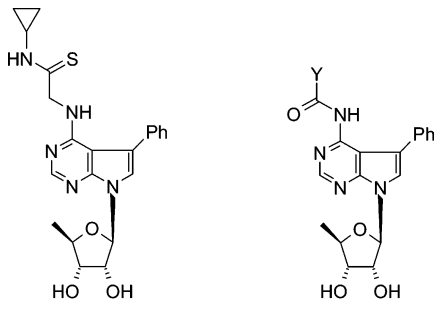


[a] (a) (i) For **14a**, **7**, **9a**, KOH, TDA-1, toluene (method A) (30%); (ii) for **14b**, **7**, **9b**, method A (40%); (iii) for **14c**, **7**, **9c**, method A; (iv) for **18a**, **12**, **9a**, method A (52%); (v) for **18b**, (1) **12**, **11**, KOH, TDA-1, acetonitrile (44%); (2) MeONa, MeOH; (3) TBS-Cl, imidazole, DMF (79%, two steps); (vi) for **18c**, (1) **13**, **10**, $BF_3{\cdot}Et_2O$, CH_3NO_2, 80 °C (50%); (2) MeONa, MeOH; (3) dimethoxypropane, DMF, HCl (60%, two steps); (b) (1) **15**, butanol, 115 °C (ca. 65%); (2) 70% TFA; (c) $PhB(OH)_2$, $(Ph_3P)_4Pd$, diglyme, 1 M Na_2CO_3 (58%); (d) (1) *t*-BuO-K^+, THF; (2) $BrCH_2CO_2Me$ (23−53%); (e) (1) *n*-propylamine or cyclopropylamine, Me_3Al, C_6H_6 reflux (ca. 50%); (2) 70% TFA or TBAF (70−94%).

dine nucleosides. This allowed for the incorporation of the amide diversity element at a later stage of the synthesis. The nucleoside **18a** was prepared from a TDA-1-mediated glycosylation between the base **12**[20] and the chloro sugar **9a**. The acyclonucleoside **18b** was prepared from **12** and the commercially available **11** in a similar manner but required a protecting group interconversion of benzoate ester to *tert*-butyldimethylsilyl ether. The pyrazolopyrimidine nucleoside **18c** was prepared from a boron trifluoride etherate-mediated glycosylation[21] between base **13**[22] and triacetate **10** followed by sodium methoxide de-esterification and finally reprotection of the vicinal hydroxy groups with an isopropylidenyl group. Pyrazolopyrimidine nucleoside **18d**, on the other hand, was prepared from readily available **17**[21,23] via a Suzuki coupling reaction[24] with phenylboronic acid. The intermediates **18a**−**d** were reacted with methyl bromoacetate in the presence of potassium *tert*-butoxide. The resulting products **19a**−**d** were converted to amides using the trimethylaluminum-mediated protocol[25] and then deprotected routinely with either TFA or TBAF to provide **16g**−**j**.

The regiochemical assignment of alkylation at N7 for **16h** is supported by ^{1}H NMR and UV/vis spectroscopies. In compounds **16c** and **16h**, the respective proton shifts of H2 and H6 are nearly identical. Also, the UV/vis λ_{max} value for **16h** [λ_{max} (methanol) 282 nm (ϵ 15 300)] agrees with that obtained for compound **2** [λ_{max} (methanol) 282 nm (ϵ 14 400)].

Several analogues were prepared with small modifications on the glycinamide functionality. The thiono analogue **20** was prepared by subjecting intermediate **16b** to reaction with the Lawesson reagent[26] followed by TFA deprotection. Additionally, urea **21** and carbamate **22** were derived from reactions of **18a** with



Scheme 4

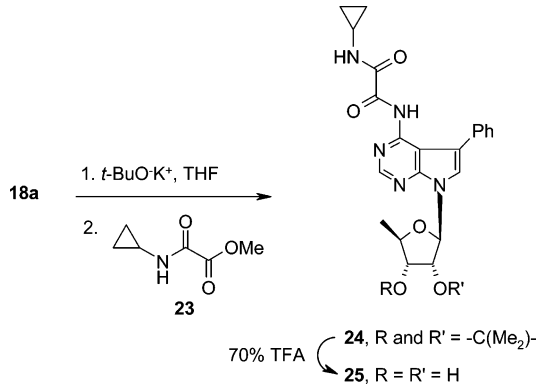
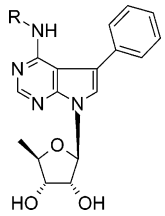


Table 1. Early SAR, Alternatives to the C4 *N*-Phenyl



compd	R	AK IC_{50} (μM)	pH 7.4 sol (μg/mL)
1[a]	Ph	0.0005	<0.05
GP3269	4-F-Ph	0.0015	<0.05
2[a]	H	0.32	
3[a]	cy-Hex	1.2	
21	C(O)NHMe	0.027	32
22	C(O)O-*n*-Pr	0.004	500
26	cy-Pr	0.38	36
27	*n*-Pr	0.10	16
28	*i*-Bu	0.14	3
29	cy-Pr-CH_2	0.045	0.1
30	$CH_2C(O)NH_2$	0.002	750

[a] See ref 1b.

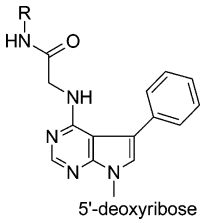
methylisocyanate and propyl chloroformate followed by TFA deprotections, respectively. Finally, treatment of compound **18a** with potassium *tert*-butoxide and amide ester **23** produced **24**, which after TFA deprotection provided oxalyl bis-amide **25** (Scheme 4). Since **24** and **25** were unstable to silica gel chromatography, they were purified by extraction and recrystallization.

Results

The AK IC_{50} values were determined using human recombinant enzyme as previously described.[1a] Compounds that showed sufficient enzyme inhibition were subjected to the rat formalin paw antinociceptive assay[9] at an oral screening dose of 20 mg/kg. The antinociceptive response is reported as the percent inhibition of the licking and biting response during the second phase of the challenge (see experimental). Compounds with >40% inhibition in the formalin paw assay were considered for further studies.

It was previously demonstrated[1b] that replacement of the C4 phenylamino group of **1** (IC_{50} = 0.0005 μM) with cyclohexylamino (i.e. **3**) resulted in a 2400-fold loss in AK inhibitory potency (Table 1). However, for smaller alkyl groups (**26**−**29**) the relative potency loss was less (e.g. **29** with IC_{50} = 0.045 μM). Furthermore, incorporation of heteroatoms in the side chain produced low nM enzyme inhibitors (**21**, **22**, and **30**), thus providing a new lead area to explore. These compounds showed significantly improved solubility in pH 7.4 buffer; however, no activity was observed in the oral pain assay.

Table 2. SAR of Glycinamide Substituent



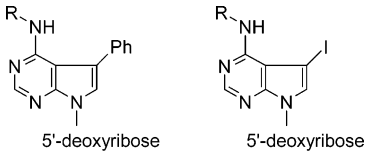
compd	R	AK IC_{50} (μM)	pH 7.4 solubility (μg/mL)	% lick/ bite inh[a]
16c	cy-Pr	0.003	32 ± 9	66
16g	*n*-Pr	0.007	38	41
31	Me	0.0008	240	35
32	Et	0.040	140	52
33	*i*-Pr	0.025	26	74
34	*i*-Bu	0.004	7	16
35	cy-Pr-CH_2	0.006	12	42
36	cy-Bu	0.0013	44	60
37	cy-pentyl	0.001	6	16
38	Ph	0.0005	<0.05	62
39	*p*-Cl-Ph	0.0002	<0.05	62
40	EtO	0.005	535	0
41	$MeO(CH_2)_2$	0.038	230	44
42	$HO(CH_2)_3$	0.016	650	4
43	$MeO(CH_2)_3$	0.002		24
44	$Me_2N(CH_2)_2$	0.30	>1000	
45	$Me_2N(CH_2)_3$	0.145	>1000	22
46	$Et_2N(CH_2)_2$	0.210	>1000	11

[a] Oral rat formalin paw assay. Inhibition of the lick/bite response during phase 2 at an oral dose of 20 mg/kg.

Additional analogues of compound **30** in which the C4 glycinamide group was substituted with a number of alkyl and aryl groups include many potent AKIs (IC_{50}s < 10 nM) with improved water solubility (7−650 μg/mL), as shown in Table 2. Importantly, several of these compounds, i.e. **16c**, **16g**, **32**, **33**, and **36**, showed good activity in the oral rat formalin paw pain assay and therefore were subjected to more detailed biological evaluation (vida infra). Aryl amides (**38** and **39**) were very potent AKIs with oral analgesic activity but were water insoluble and displayed toxicity in hepatotoxicity assays, so they were not pursued further. Water solubility was improved to >1 mg/mL by incorporation of a tertiary amine in the molecules, as seen with **44**-**46**, but these were poor enzyme inhibitors. The cyclopropyl- and phenylglycinamide moieties of analogues **16c** and **38** were incorporated into other molecules (Tables 3 and 4) to evaluate the effect on biological activity when further changes were made to the base and sugar components.

Further variations on the C4-glycinamide substituent show how small structural changes on this region of the molecule can have profound effects on enzyme inhibition, water solubility, and analgesic activity (Table 3). Substitution of sulfur for the amide carbonyl oxygen (**20**) increased enzyme inhibitor potency 6-fold, IC_{50} = 0.0005 μM, but water solubility decreased, and antinociception in the formalin paw assay was not significant. Likewise, with a carbonyl group added as in the oxalyl bis-amide example **25**, similar effects were seen. However, methyl substitution in the α-position of the amide (**47** and **48**) and on the amide nitrogen (**49**) decreased the inhibitory

Table 3. Miscellaneous Glycinamide Analogue SAR

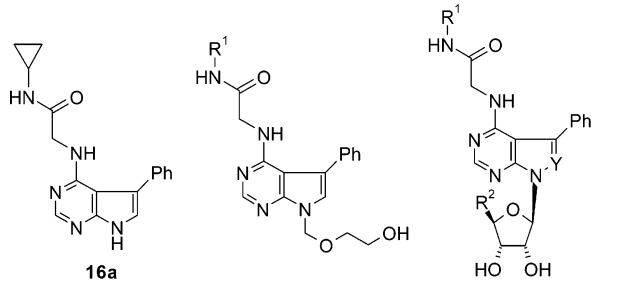


20, **25**, **47**-**50** **51**

compd	R	AK IC_{50} (μM)	pH 7.4 solubility (μg/mL)	% lick/ bite inh[a]
20	$CH_2C(S)NH$-cy-Pr	0.0005	8	22
25	C(O)C(O)NH-cy-Pr	0.0013	17	17
47	CH(*S*-Me)C(O)NH-cy-Pr	0.53	480	
48	CH(*R*-Me)C(O)NH-cy-Pr	2.5		
49	$CH_2C(O)N$(Me)-cy-Pr	0.46	105	
50	$CH_2CH_2C(O)NH_2$	2.5		
51	$CH_2C(O)NH$-cy-Pr	0.88	5	

[a] Oral rat formalin paw assay. Inhibition of the lick/bite response during phase 2 at an oral dose of 20 mg/kg.

Table 4. SAR of Sugar Component



16a **16h**, **52** **16d**, **16e**, **16i**, **16j**, **53**, **54**

cmpd	R^1	R^2	Y	AK IC_{50} (μM)	pH 7.4 solubility (μg/mL)	% lick/ bite inh[a]
16a				>10		
16h	cy-Pr			1.7	270	
52	*p*-Cl-Ph			0.060	<0.05	31
16d	cy-Pr	CH_2OH	CH	0.003	76	21
16e	cy-Pr	H	CH	0.020	280	17
53	Ph	H	CH	0.0006	0.5	37
16i	cy-Pr	CH_3	N	0.006	620	7
16j	cy-Pr	CH_2OH	N	0.010	370	6
54	Ph	CH_2OH	N	0.0009	7	−10

[a] Oral rat formalin paw assay. Inhibition of the lick/bite response during phase 2 at an oral dose of 20 mg/kg.

potency by 100−1000-fold when compared to that of **16c**. Additionally, as demonstrated with **50**, addition of one methylene group between the amide and N^4 greatly decreased inhibitor potency (>1000-fold decrease compared to **30**, Table 1), which emphasizes the specificity of the binding interaction with the glycinamide substituent. Last, the 5-iodo analogue **51** is a 300-fold weaker enzyme inhibitor than the 5-phenyl compound **16c**, which parallels the results previously seen with the 5-iodo analogue of **1**.[1b]

Several variations in the sugar and base component of the molecule were examined on the basis of SAR previously discovered in the diaryltubercidin series[1b,3a,6a,27] (see Table 4). The aglycon **16a** itself was devoid of AK inhibitory activity, indicating the importance of the sugar moiety to compound affinity. Incorporation of a sugar fragment as in **16h** and **52** resulted in a potent enzyme inhibitor only with the *p*-chlorophenylamide (**52**, IC_{50} = 0.060 μM). The ribose and erythrose analogues **16d**, **16e**, and **53** as well as the pyrazolopyrimidine analogues **16i**, **16j**, and **54** were all potent enzyme inhibitors. Many also had satisfactory water solubility, but all were devoid of notable analgesic activity.

Table 5. Activity Summary of **16c** in Advanced Assays[a]

- Human recombinant AK IC_{50} = 0.003 μM
- Water solubility: 32+/-9 μg/mL at pH 7.4
- Oral fasted rat formalin paw pain assay ED_{50} = 2.5 mg/kg
- Oral rat mechanical hyperalgesic assay ED_{50}s (mg/kg):

	3h	4h	5h	6h
Prophylactic	2.6	3.8	<50%	ND
Therapeutic	1.6	4.1	6.5	<50%

- Oral rat rotorod assay ED_{50}s (mg/kg):

0.5 h	1h	2h	5h
>300	>300	102	62

- Oral marmoset monkey bradykinin-induced licking assay ED_{50} = 0.9 mg/kg
  Emesis (3/4) and ataxia observed at 10 but not at 1 or 0.1 mg/kg
- OBAV = 128+/-36% (rat) and 278+/-109% (dog)
- $T_{1/2}$ (h) = 0.3+/-0.1 (rat early phase), 5.2+/-0.4 (rat late phase) and 2.0+/-0.2 (dog)
- $T_{1/2}$ (h) = 3.3 in human liver microsomes
- No rat liver toxicity @ 75 μmol/kg iv (2 doses separated by 12 h)
- Tolerance observed in oral rat formalin paw assay after 30 mg/kg b.i.d. dosing for 4 days
- Deaths (3 of 6) observed in oral rat formalin paw assay after 60 mg/kg b.i.d. dosing for 4 days

[a] Additional experimental data containing dose−response graphs for the rat formalin paw assay, rat mechanical hyperalgesic assay, rat rotorod assay, monkey bradykinin-induced licking assay, full PK data in rats and dogs, and liver panel data can be found in the Supporting Information.

On the basis of the in vitro and in vivo potencies, as well as the solubility data, **16c** was chosen for additional studies for evaluation as a lead candidate for the treatment of pain. Results of these studies are summarized in Table 5 (see Supporting Information for detailed experimental results). The ED_{50} in the fasted oral rat formalin paw assay for pain was 2.5 mg/kg. Additionally, **16c** was prophylactically and therapeutically effective in an assay of inflammatory pain, the rat carrageenan induced mechanical hyperalgesic test[28] (ED_{50}s 1.6−6.5 mg/kg, 3−5 h after carrageenan injection). Furthermore, **16c** showed a reasonable therapeutic window with respect to motor side effects. The oral rat rotarod ED_{50} was 62 to >300 mg/kg at different time intervals, demonstrating a 24- to >120-fold separation of the side effects of ataxia and sedation from antinociception.

Unlike the rat, the marmoset monkey is sensitive to ataxia, sedation, and emesis, which are some of the common side effects of CNS drugs in man. In an oral bradykinin-induced pain assay in the marmoset monkey,[29] **16c** was more potent (ED_{50} = 0.9 mg/kg po) than morphine and demonstrated a 10-fold larger therapeutic index with respect to CNS side effects. Administration of **16c** induced emesis in three of four monkeys as well as ataxia in all monkeys at 10 mg/kg. However, these side effects were not observed at the 1 or 0.1 mg/kg dose. It is noteworthy that in this model, morphine, a standard pain therapy, showed an ED_{50} = 0.33 mg/kg sc. Morphine-treated monkeys began to show ataxia at 0.1 mg/kg sc (one of three).

Compound **16c** demonstrated excellent oral bioavailability in rats and dogs, equaling 128 ± 36% and 278 ±

109%, respectively. The early elimination phase was longer in dogs ($T_{1/2} = 2.0 \pm 0.2$ h) than in rats ($T_{1/2} = 0.3 \pm 0.1$ h). Moreover rats had a late phase elimination process ($T_{1/2} = 5.2 \pm 0.4$ h). In human liver microsomal studies, the half-life of the compound was found to be 3.3 h. Furthermore, **16c** did not cause elevation in liver enzymes in the rat liver function assay.

In chronic dosing studies in the rat formalin paw assay (30 mg/kg b.i.d. 4 days), tolerance to **16c** was apparent, as evidenced by a loss of analgesic activity. Additionally in these studies, deaths of three out of six experimental animals was associated with prolonged administration of the compound (60 mg/kg b.i.d. 4 days). The exact nature and cause of this toxicity were not examined in detail.

Discussion

The diaryltubercidin AKIs, as exemplified by the prototype compound **1**, are very potent enzyme inhibitors and are active in assays for epilepsy,[1] stroke,[2] inflammation,[3] acute myocardial infarction,[4] and analgesia[6a,b] but they are limited by poor water solubility (**1**, water solubility < 0.05 μg/mL). Hypothesizing that the lack of solubility was related to the C4- and C5-aryl rings forming a stable stacking arrangement in the solid state, we sought to eliminate this effect by substituting a flexible chain containing heteroatoms for the C4-arylamino group. However, preliminary work had indicated that both the C4- and C5-aryl groups were required to produce a potent AKI. Further investigation revealed that branched alkylamino C4-substituted compounds such as **29** were potent inhibitors ($IC_{50} = 0.045$ μM). Suprisingly, acylamino (i.e. **22**) and glycinamide (i.e. **30**) C4-substituted analogues were found to be very potent AKIs (IC_{50}s = 0.004 and 0.002 μM, respectively). Additionally, these substituents served to make the compounds more water-soluble, as desired (**30** water solubility = 750 μg/mL).

Examining the effects of various small changes in the glycinamide group revealed several key features that are important for the inhibition of AK (see Table 3). Compared to **16c** (AK $IC_{50} = 0.003$ μM), the tertiary amide **49** is a 200-fold weaker AKI, while the thioamide **20** is more potent, indicating that the amide NH and not the carbonyl may have a hydrogen bond with the active site of AK. The relative geometric orientation of the glycinamide group is crucial to potency, since α-methyl substituents, as in compounds **47** and **48**, decrease enzyme inhibition, while the α-oxo substituent of **25** maintains activity comparable to compound **16c**. This is most dramatically demonstrated by the 1000-fold decrease in enzyme potency that results from adding one methylene group to this substituent (see **50**).

Potent AKI activity of various glycinamide substituted compounds can be explained by a model wherein a hydrogen bond forms between the amide NH group and AK (see Figure 1). X-ray crystallographic structures of adenosine complexed to human and parasitic AK show two water molecules forming bridges from protein residues to the N^6 and N7 of adenosine (see Figure 1A).[30] We propose that the glycinamide group replaces the water bridge "above" N^4 (equivalent to N^6 in adenosine) and, more specifically, that the amide NH of this substituent replaces the H-bond to either Thr173 or Phe170 (as defined for human AK) previously held by Wat416 (see Figure 1B).

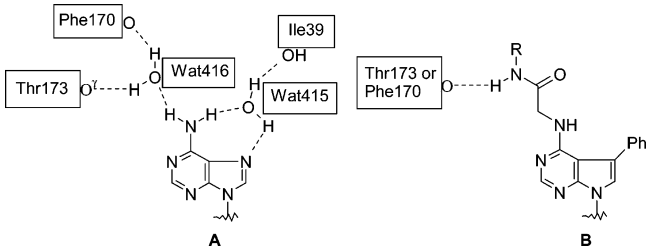


Figure 1. Structure **A** shows the hydrogen bonding of adenosine with water molecules bridging to residues in the active site of AK as defined by the X-ray crystal structure determined for human AK (see ref 30a). Structure **B** shows how the glycinamide functionality replaces one of the water bridges to the active site residue Thr173 or Phe170.

The glycinamide C4-substituted analogues have increased water solubility, but combining this with antinociceptive activity proved to be a significant challenge. While the solubility of some analogues approached 1 mg/mL, those active in the oral rat formalin paw assay of antinociception, **16c**, **16g**, **32**, **33**, and **36**, were less soluble, 26−140 μg/mL. This solubility is in the range predicted necessary for successful oral drug candidates.[12,13] A few compounds that were inactive in the formalin paw assay, **31** (240 μg/mL), **40** (535 μg/mL), **16d** (76 μg/mL), **16j** (370 μg/mL), were only slightly different in structure but were similar in water solubility and enzyme potency. There may be a threshold relationship between maximum water solubility and active brain penetration for these compounds. Alternatively, rapid metabolism may be responsible for the inactivity of some of these compounds. Detailed studies were not pursued. The five CNS-active compounds are all 5′-deoxypyrrolopyrimidine ribosides with small *N*-alkylglycinamide C4-substitutents. More than 100 analogues (some of these to be reported elsewhere) were evaluated where changes were made to the amide, base, and sugar moieties of the lead molecule **16c**, but combined water solubility and analgesic properties were limited to only these five compounds.

Conclusion

This investigation demonstrated that replacing a phenyl group in the diaryltubercidin series (e.g. **1**) with a flexible side chain containing several heteroatoms, i.e., the glycinamide group, can increase water solubility while maintaining AK enzyme inhibitory potency and oral analgesic activity. This was unexpected on the basis of previous SAR. Extensive analogue studies in which variations were made to the base, sugar, and glycinamide group of this nucleoside series revealed that oral analgesic activity coupled with suitable water solubility (close to or better than 50 μg/mL)[12,13] was limited to a narrow group of 5′-deoxypyrrolopyrimidine ribosides, i.e., **16c**, **16g**, **32**, **33**, and **36**. Compound **16c** was evaluated in detail and demonstrated analgesic effects in several animal models (e.g. oral marmoset monkey bradykinin-induced licking assay of acute pain; ED_{50} = 0.9 mg/kg). Further investigations were discontinued when tolerance and death were observed in chronic evaluations in the oral rat formalin paw assay. The exact cause of these adverse effects observed is as yet unknown.

Experimental Section

General Methods. Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature in a desiccator, and all reactions were carried out under an atmosphere of nitrogen. Anhydrous solvents were purchased from Aldrich and stored over 4A molecular seives. Thin-layer chromatography was performed with EM Science silica gel 60 F_{254} aluminum-backed sheets. Flash chromatography was performed on 230−400 mesh EM Science silica gel 60. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR were obtained on a Varian Gemini-200 operating at 200 MHz, and spectra were recorded in units δ with tetramethylsilane (δ 0.000), $CD_2HS(O)CD_3$ (δ 2.504), or $CDCl_3$ (δ 7.260) as reference line internal standards. UV spectra (200−350 nM) of methanolic solutions were recorded on a Kontron Uvikon 860. Analytical HPLC was performed on a 4.6 × 250 mm YMC ODS-AQ 5 μm column eluted with a 0.1% aqueous AcOH/MeOH gradient with the UV/vis detector at 280 nM. C, H, N microanalyses were performed by NuMega Resonance Labs, Inc., San Diego, CA, or by Robertson Microlit Laboratories, Inc., Madison, NJ. Low-resolution mass spectral (LRMS) analyses were performed by Mass Consortium, San Diego, CA.

2-Amino-4-phenyl-1*H*-pyrrole-3-carboxylic Acid Amide (5). To **4**[14] (100 g, 0.55 mol) was added sulfuric acid (200 mL) at a rate that brought the temperature up to ca. 80 °C. The reaction was heated to 100 °C and stirred for 1 h, and the solution was cooled with an ice bath and then poured onto a mixture of 2 L of ice and 1 L of EtOAc. To this mixture under cooling in an ice bath was added slowly with stirring an ice cold solution of 300 g of NaOH dissolved in 300 mL of water (**Warning:** *exothermic caustic mixture*) until pH 7.5 resulted. The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 1 L). The combined EtOAc extracts were washed with brine, dried ($MgSO_4$), and concentrated to a volume of 500 mL. Filtration provided 65.9 g of **5**. Further concentration of the filtrate to ca. 100 mL and then dilution with 400 mL of ether provided an additional 19.4 g for a total of 85.3 g (76%) of **5** as a brick-colored solid: R_f 0.45 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 5.84 (br s, 2H, NH_2), 6.11 (d, 1H, J = 2 Hz, H5), 7.2−7.5 (m, 5H, Ar Hs), 10.19 (br s, 1H, NH).

5-Phenylpyrrolo-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one (6). Compound **5** (52 g, 0.26 mol), 300 mL of DMF, triethylorthoformate (129 mL, 0.78 mol), and *p*-toluenesulfonic acid monohydrate (1.71 g, 9 mmol) were combined and stirred at room temperature for 16 h. The solvents were evaporated and the residue suspended in 200 mL of MeOH. The resulting brown solid was collected by filtration and washed with MeOH (3 × 50 mL) to provide 39.3 g (72%) of **6** sufficiently pure for the next procedure: R_f 0.51 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 6.98 (d, 1H, J = 3 Hz, H6), 7.27 (tt, 1H, J = 7, 1 Hz, *p*-H), 7.41 (td, 2H, J = 7, 1 Hz, *m*-Hs), 7.61 (dt, 2H, J = 7, 1 Hz, *o*-Hs), 8.28 (d, 1H, J = 1 Hz, H2), 11.02 (br s, 1H, NH), 11.94 (br s, 1H, NH).

4-Chloro-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (7). A mixture of **6** (55.0 g, 0.26 mol) and 250 mL of $POCl_3$ was heated at 120°C for 2.5 h. The reaction mixture was allowed to cool to room temperature, and the contents were poured slowly over crushed ice. EtOAc (2 L) was added and the mixture stirred vigorously. After separating the layers, the aqueous layer was neutralized with sodium bicarbonate and extracted with EtOAc (2 × 1 L). The organic extracts were combined and evaporated, and the resulting residue was suspended in 500 mL of ice water. The resulting blue solid was collected by filtration and dried under a high vacuum at room temperature to provide 50 g (84%) of **7**: R_f 0.61 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 7.1−7.3 (m, 6H, Ar Hs and H6), 8.31 (s, 1H, H2), 11.26 (br s, 1H, NH).

4-Chloro-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-D-ribofuranosyl)-5-phenylpyrrolo[2,3-*d*]pyrimidine (14a) was prepared as a tan amorphous solid from chloro-sugar **9a**[1a] and the potassium salt of **7** as described for **18a**. **14a** (30% yield): R_f 0.47 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 1.25 (d, 3H, J = 6 Hz, 5′-Me), 1.33 (s, 3H, IP-Me), 1.55 (s, 3H, IP-Me), 4.08 (quintet, 1H, J = 6 Hz, H4′), 4.74 (t, 1H, J = 6 Hz, H3′), 5.48 (dd, 1H, J = 6, 2 Hz, H2′), 6.61 (d, 1H, J = 2 Hz, H1′), 7.2−7.5 (m, 6H, Ar Hs and H6), 8.29 (s, 1H, H2).

7-(5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)-4-chloro-5-phenylpyrrolo[2,3-*d*]pyrimidine (14b) was prepared as a tan amorphous solid from chloro sugar **9b**[1b] and the potassium salt of **7** as described for **18a**. **14b** (40% yield): ^{1}H NMR (DMSO-d_6) δ −0.1 to 0.0 (m, 6H, TBDMS−CH_3s), 0.82 (s, 9H, *t*-Bu), 1.32 (s, 3H, IP-Me), 1.54 (s, 3H, IP-Me), 3.67 (dd, 1H, J = 10, 6 Hz, H5′), 3.78 (dd, 1H, J = 10, 6 Hz, H5′), 4.22 (dt, 1H, J = 6, 3 Hz, H4′), 4.92 (dd, 1H, J = 7, 3 Hz, H3′), 5.30 (dd, 1H, J = 7, 3 Hz, H2′), 6.42 (d, 1H, J = 3 Hz, H1′), 7.3−7.5 (m, 5H, Ar Hs), 7.93 (s, 1H, H6), 8.71 (s, 1H, H2).

4-Chloro-7-(2,3-*O*-isopropylidenyl-β-d-erythrofuranosyl)-5-phenylpyrrolo[2,3-*d*]pyrimidine (14c) was prepared as a tan amorphous solid from chloro sugar **9c**[6a] and the potassium salt of **7** as described for **18a**. **14c**: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3H, Me), 1.60 (s, 3H, Me), 4.26 (d, 1H, J = 10 Hz, H4′), 4.31 (dd, 1H, J = 10, 3 Hz, H4′), 5.31 (dd, 1H, J = 6, 3 Hz, H3′), 5.58 (d, 1H, J = 6 Hz, H2′), 6.11 (s, 1H, H1′), 7.27 (s, 1H, H6), 7.35−7.55 (m, 5H, Ar Hs), 8.63 (s, 1H, H2).

Glycine Cyclopropylamide (15). A mixture of *N*-carbobenzyloxyglycine (10.0 g, 47.8 mmol), cyclopropylamine (5.0 mL, 71.8 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 13.8 g, 71.8 mmol) in 200 mL of CH_2Cl_2 was stirred at room temperature for 16 h; washed with water, 5% aqueous HCl, 1 M aqueous $NaHCO_3$, and brine; dried (Na_2SO_4); and evaporated to provide 10.3 g (87%) of *N*-carbobenzyloxyglycine cyclopropylamide as a white solid: R_f 0.45 (10:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 0.35−0.4 (m, 2H, cyclopropyl Hs), 0.55−0.65 (m, 2H, cyclopropyl Hs), 2.5−2.7 (m, 1H, cyclopropyl H), 3.53 (d, 2H, J = 6 Hz, C2 Hs), 5.02 (s, 2H, benzylic Hs), 7.25−7.45 (m, 5H, Ar Hs), 7.92 (d, 1H, J = 3 Hz, amide NH).

A mixture of *N*-carbobenzyloxyglycine cyclopropylamide (10.3 g, 41.5 mmol) and 10% Pd/C (2.0 g) in 120 mL of MeOH was shaken under 30 psi of H_2 pressure for 16 h and then filtered through a pad of Celite and evaporated to provide 4.7 g (100%) of the title compound **15** as a colorless oil: R_f 0.07 (10:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 0.3−0.45 (m, 2H, cyclopropyl Hs), 0.5−0.65 (m, 2H, cyclopropyl Hs), 2.5−2.7 (m, 1H, cyclopropyl H), 3.00 (s, 2H, C2 Hs), 7.8 (br s, 1H, amide NH).

The cyclopropylamides of D- and L-alanine, glycine phenylamide, and the 3-hydroxypropylamide[31] of glycine were prepared in the same manner. These compounds were used for the preparation of the final compounds **16a**, **16c**−**e**, **42**, **47**, and **48**.

4-*N*-(*N*-Cyclopropylcarbamoylmethyl)amino-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (16b). General Method for Amine Addition Reactions on 4-Chloropyrrolopyrimidines 7, 14a, 14b, and 14c. A mixture of 4-chloropyrrolopyrimidine **14a** (2.50 g, 6.5 mmol) and glycine cyclopropylamide (**15**) (3.70 g, 32 mmol) was refluxed in 120 mL of *n*-butanol (bp 118 °C) for 30 h and then cooled to room temperature. The resulting solid was collected and washed with *n*-butanol, triturated with water, and dried under vacuum to provide 1.92 g (64%) of the title compound **16b** as a white solid: R_f 0.1 (1:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 0.3−0.45 (m, 2H, cyclopropyl Hs), 0.5−0.65 (m, 2H, cyclopropyl Hs), 1.28 (d, 3H, J = 7 Hz, 5′-CH_3), 1.31 and 1.53 (s each, 3H each, IP-CH_3s), 2.5−2.7 (m, 1H, cyclopropyl H), 4.02 (d, 2H, J = 4 Hz, amide α-CH_2), 4.14 (dq, 1H, J = 7, 4 Hz, H4′), 4.74 (dd, 1H, J = 6, 4 Hz, H3′), 5.32 (dd, 1H, J = 6, 2 Hz, H2′), 5.99 (t, 1H, J = 4 Hz, C4 NH), 6.24 (d, 1H, J = 2 Hz, H1′), 7.3−7.6 (m, 6H, Ar Hs and H6), 8.13 (d, 1H, J = 4 Hz, amide NH), 8.25 (s, 1H, H2).

In this manner, compounds **42** and **16a** were prepared. Also the 2′,3′-*O*-isopropylidenyl or otherwise protected analogues of the following final compounds were prepared: **16d**, **16e**, **26**−**31**, and **47**−**50**. Finally, the 2′,3′-*O*-isopropylidenyl-protected derivative of **51** was prepared from known compound 4-chloro-

5-iodo-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine.[1a]

4-*N*-(*N*-Cyclopropylcarbamoylmethyl)amino-5-phenyl-7-(5-deoxy-β-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (16c). General Method for TFA Deprotection of 2′,3′-*O*-Isopropylidenyl Nucleoside Derivatives. A mixture of compound **16b** (2.08 g, 4.5 mmol) in 10 mL of 70% aqueous TFA was stirred for 2 h at room temperature and concentrated by rotary evaporation at 25 °C. It was diluted with water and neutralized with aqueous 2 M NaOH at 5 °C. The resulting suspension was sonicated for 5 min and filtered. The collected solid was washed with water and dried at 100 °C /2 mm for 2 h to provide 1.79 g (94%) of the title compound **16c** as a white solid: mp 213−214 °C; R_f 0.1 (10:1 CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6) δ 0.3−0.4 (m, 2H, cyclopropyl Hs), 0.5−0.65 (m, 2H, cyclopropyl Hs), 1.28 (d, 3H, J = 6 Hz, 5′-CH_3), 2.5−2.7 (m, 1H, cyclopropyl methine), 3.8−4.0 (m, 2H, H4′ and H3′), 4.02 (d, 2H, J = 4 Hz, amide α-CH_2), 4.48 (q, 1H, J = 5 Hz, H2′), 5.12 (d, 1H, J = 5 Hz, OH), 5.37 (d, 1H, J = 6 Hz, OH), 5.96 (t, 1H, J = 4 Hz, C4 NH), 6.12 (d, 1H, J = 5 Hz, H1′), 7.3−7.6 (m, 6H, Ar Hs and H6), 8.13 (d, 1H, J = 3 Hz, amide NH), 8.23 (s, 1H, H2). Anal. ($C_{22}H_{25}N_5O_4$.0.75 H_2O) C, H, N.

In this manner the following compounds were prepared from 2′,3′-*O*-isopropylidenyl (IP-protected) precursors: **16d**, **16e**, **16g**, **16i**, **16j** , **26**−**41**, **43**−**51**, **53**, and **54**.

4-Amino-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (18a). General Method for Tris(2-(2-methoxyethoxy)ethyl)amine (TDA-1)-Mediated Glycosylation. A mixture of pyrrolopyrimidine **12**[20] (5.00 g, 23.8 mmol), KOH (3.13 g, 47.6 mmol, freshly ground to a powder with a hot mortar and pestle), and TDA-1 (7.7 mL, 24.0 mmol) in 45 mL of acetonitrile was stirred for 2 h at room temperature to form a yellow suspension. In a separate flask, a mixture of 38 mL of toluene, 13 mL of acetonitrile, and DMF (3.7 mL, 47.6 mmol) was chilled to −30 °C and oxalyl chloride (4.15 mL, 47.6 mL) was added slowly. A suspension formed with gas evolution. The temperature was raised to −15 °C and the mixture stirred for 5 min. A solution of sugar **8a**[1a] (8.29 g, 47.6 mmol) in 10 mL of toluene was added slowly to this via cannula needle while the temperature was maintained between −15 and −12 °C, and the resulting mixture was stirred an additional 20 min at this temperature range. Then triethylamine (9.28 mL, 66.6 mmol) was added, the temperature raised to −3 °C, and the mixture stirred a further 15 min. This suspension containing chloro sugar **9a** was filtered through Celite directly into the mixture containing the above prepared potassium salt of heterocycle **12**. The Celite was rinsed with toluene (2 × 5 mL). The temperature was raised to room temperature and the mixture stirred for 16 h. The solution was diluted with EtOAc; washed with water, 1 M HCl, water, and brine; dried ($MgSO_4$); and evaporated. The residue obtained was subjected to flash chromatography on SiO_2 eluting with hexane/EtOAc mixtures of 2:1, 1.25:1, and 1:1 which provided 3.19 g of **18a**. Continued elution provided an impure fraction that was rechromatographed to provide an additional 1.56 g for a total of 4.74 g (52%) of **18a** as a light yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, J = 7 Hz, 5′-CH_3), 1.31 and 1.53 (s each, 3H each, IP-CH_3s), 4.14 (dq, 1H, J = 7, 3 Hz, H4′), 4.72 (dd, 1H, J = 7, 3 Hz, H3′), 5.32 (dd, 1H, J = 7, 3 Hz, H2′), 6.2 (br s, 2H, NH_2), 6.23 (d, 1H, J = 3 Hz, H1′), 7.3−7.6 (m, 6H, Ar Hs and H6), 8.18 (s, 1H, H2).

4-Amino-5-phenyl-7-(*tert*-butyldimethylsilyloxyethoxymethyl)pyrrolo[2,3-*d*]pyrimidine (18b). Step 1. To compound **12** (10.00 g, 47.5 mmol) and powdered KOH (4.00 g, 71.3 mmol) was added 40 mL of CH_3CN and TDA-1 (15.3 mL, 47.5 mmol), and the resulting mixture stirred for 1 h at room temperature when it became brown and homogeneous. The mixture was chilled to 3 °C and then chloride (**11**) (14.7 mL, 71.3 mmol) was added. Then the mixture was stirred at room temperature for 16 h; diluted with EtOAc; washed with water, 1 N HCl, water, and brine; dried ($MgSO_4$); and evaporated. The residue was subjected to flash chromatography eluting with CH_2Cl_2/MeOH mixtures of 50:1, 35:1, 25:1, and 20:1 to provide 7.2 g (39%) of the desired product as a solid. Impure fractions were rechromatographed to provide an additional 1.0 g (5%) for a total of 8.2 g (44%) of 4-amino-5-phenyl-7-(benzoyloxyethoxymethyl)pyrrolo[2,3-*d*]pyrimidine: ^{1}H NMR ($CDCl_3$) δ 3.84 (t, 2H, J = 5 Hz, OCH_2), 4.28 (t, 2H, J = 5 Hz, OCH_2), 5.49 (br s, 2H, NH_2), 5.70 (s, 2H, NCH_2O), 7.10 (s, 1H, H6), 7.3−7.6 (m, 8H, Ph groups), 7.97 (d, 2H, J = 8 Hz, benzoate *o*-Hs), 8.32 (s, 1H, H2).

Step 2. A solution prepared by dissolving sodium metal (241 mg, 10.5 mmol) in 100 mL of methanol was added to the ester 4-amino-5-phenyl-7-(benzoyloxyethoxymethyl)pyrrolo[2,3-*d*]-pyrimidine (8.11 g, 20.9 mmol) and the resulting mixture stirred for 1 h at room temperature. Then it was diluted with 400 mL of CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated to 6.5 g of intermediate alcohol which was used without further purification in the next reaction. This residue was mixed with *tert*-butyldimethylsilyl chloride (TBS−Cl) (3.80 g, 25.2 mmol) and imidazole (1.72 g, 25.2 mmol) in 100 mL of DMF for 14 h at room temperature. Then it was diluted with 500 mL of ether, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to flash chromatography eluting with CH_2Cl_2/MeOH mixtures of 50:1, 35:1 and 25:1 to provide 6.64 g (79% for 2 steps) of the title compound (**18b**) as a hard foam: ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H, $SiMe_2$), 0.86 (s, 9H, *t*-Bu), 3.60 (dt, 2H, J = 5, 1 Hz, OCH_2), 3.74 (dt, 2H, J = 5, 1 Hz, OCH_2), 5.15 (br s, 2H, NH_2), 5.68 (s, 2H, NCH_2O), 7.12 (s, 1H, H6), 7.3−7.5 (m, 5H, Ph), 8.35 (s, 1H, H2).

4-Amino-1-(5-deoxy-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)-3-phenylpyrazolo[3,4-*d*]pyrimidine (18c). Step 1. A mixture of 5-deoxy-2,3-*O*-isopropylidenylribose (**8a**) (5.00 g, 28.7 mmol) in 20 mL of 0.1 N H_2SO_4 was stirred at 80 °C for 4 h and then cooled to 5 °C and neutralized to pH 7 with 2 M NaOH. The mixture was frozen and lyophilized to produce an oil. To this, 50 mL of acetonitrile was added, which caused a white solid to form. The mixture was dried further by addition of $MgSO_4$ and then it was filtered and the filtrate evaporated to provide 5 g of crude 5-deoxyribose[1a] which was used without further purification: TLC R_f = 0.26 (10:1 CH_2Cl_2/MeOH).

Step 2. This residue of crude 5-deoxyribose (5 g) was dissolved in 60 mL of acetonitrile, and 4-*N*,*N*-(dimethylamino)-pyridine (1.75 g, 14.4 mmol), triethylamine (20 mL, 144 mmol), and acetic anhydride (12.2 mL, 129 mmol) were added. This was stirred for 14 h at room temperature and then methanol (3.6 mL, 86 mmol) was added. Stirring was continued at room temperature for 8 h, the solvent evaporated, and then the residue dissolved in a mixture of ether and water. The layers were separated and the ether layer washed with water and brine, dried ($MgSO_4$), and evaporated to provide 6.2 g (83%) of 5-deoxyribose-1,2,3-triacetate[18] (**10**) as a mixture of 1-α and 1-β isomers which were used without further purification: TLC R_f = 0.73 (major) and 0.65 (minor) (1:1 hexane/EtOAc).

Step 3. To a suspension of the sugar **10** (6.2 g, 23.8 mmol) and 4-amino-3-phenylpyrazolo[3,4-*d*]pyrimidine (**13**)[22] (4.57 g, 21.7 mmol) in refluxing nitromethane (80 °C) was added boron trifluoride etherate (2.7 mL, 21.7 mmol) slowly over a period of 15 min, and a clear orange solution resulted. After 30 min at 80 °C, more boron trifluoride etherate (0.54 mL, 4.4 mmol) was added. After an additional 1.5 h at 80 °C, more boron trifluoride etherate (0.27 mL, 2.2 mmol) was added. Heating was continued for 2 h, the mixture was cooled to room temperature, and triethylamine (4.5 mL, 32.6 mmol) was added. The mixture was concentrated by rotary evaporation, diluted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to flash chromatography eluting with CH_2Cl_2 and then CH_2Cl_2/methanol mixtures of 100:1 and 50:1, which eluted 4.70 g (53%) of 4-amino-1-(5-deoxy-2,3-diacetoxy-β-D-ribofuranosyl)-3-phenylpyrazolo[3,4-*d*]pyrimidine as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.34 (d, 3H, J = 6 Hz, 5′-CH_3), 2.08 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 4.29 (quintet, 1H, J = 6 Hz, H4′), 5.39 (t, 1H, J = 6 Hz, H3′), 5.90 (dd, 1H, J = 6, 3 Hz, H2′), 6.41 (d, 1H, J = 3 Hz, H1′), 7.5−7.7 (m, 5H, Ph), 8.31 (s, 1H, H6).

Step 4. A 1 M sodium methoxide solution was prepared by mixing sodium metal (508 mg, 22 mmol) with 22 mL of dry

methanol. The compound 4-amino-1-(5-deoxy-2,3-diacetoxy-β-D-ribofuranosyl)-3-phenylpyrazolo[3,4-*d*]pyrimidine (4.70 g, 11.4 mmol) was dissolved in 30 mL of methanol, and 2.3 mL of 1 M sodium methoxide in methanol was added. After stirring for 2 h at room temperature, the mixture was diluted with 200 mL of CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and evaporated to provide 3.11 g of 4-amino-1-(5-deoxy-β-D-ribofuranosyl)-3-phenylpyrazolo[3,4-*d*]pyrimidine, which was used in the next reaction without further purification: ^{1}H NMR (DMSO-d_6) δ 1.11 (d, 3H, J = 6 Hz, 5′-CH_3), 3.85 (quintet, 1H, J = 6, 4 Hz, H4′), 3.98 (q, 1H, J = 6 Hz, H3′), 4.46 (dt, 1H, J = 6, 3 Hz, H2′), 4.99 (d, 1H, J = 6 Hz, 3′-OH), 5.30 (d, 1H, J = 6 Hz, 2′-OH), 6.04 (d, 1H, J = 3 Hz, H1′), 7.3−7.5 (m, 5H, Ph), 8.16 (s, 1H, H6).

Step 5. To a solution of 4-amino-1-(5-deoxy-β-D-ribofuranosyl)-3-phenylpyrazolo[3,4-*d*]pyrimidine (3.00 g, 10.2 mmol) and dimethoxypropane (12.6 mL, 102 mmol) in 30 mL of DMF was added methanolic HCl until the solution pH was equal to 1. After stirring at room temperature for 2 h, the mixture was diluted with CH_2Cl_2; washed with aqueous sodium carbonate, water, and brine; dried ($MgSO_4$); and evaporated. The residue was subjected to flash chromatography eluting with hexane/EtOAc mixtures of 2:1 and 1:1 to provide 2.2 g of compound **18c** as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.14 (d, 3H, J = 6 Hz, 5′-CH_3), 1.19 and 1.39 (s each, 3H each, IP-CH_3s), 4.19 (dq, 1H, J = 2, 6 Hz, H4′), 4.66 (dd, 1H, J = 2, 6 Hz, H3′), 5.32 (dd, 1H, J = 2, 6 Hz, H2′), 6.24 (d, 1H, J = 2 Hz, H1′), 7.4−7.6 (m, 5H, Ph), 8.17 (s, 1H, H6).

4-Amino-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)-3-phenylpyrazolo[3,4-*d*]pyrimidine (18d). A mixture of 4-amino-1-(2,3-*O*-isopropylidenyl-β-D-ribofuranosyl)-3-bromopyrazolo[3,4-*d*]pyrimidine[21,23] (15.00 g, 42.8 mmol), TBS−Cl (6.78 g, 51.4 mmol), and imidazole (3.65 g, 53.5 mmol) in 250 mL of DMF was stirred at room temperature for 16 h. Then the mixture was diluted with ether, washed with water and brine, dried ($MgSO_4$), and evaporated to provide 21.15 g (106%) of compound **17** as a solid which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 6H, Me_2Si), 0.87 (s, 9H, *t*-Bu), 1.40 (s, 3H, IP-CH_3), 1.59 (s, 3H, IP-CH_3), 3.60 (dd, 1H, J = 10, 6 Hz, H5′), 3.70 (dd, 1H, J = 10, 6 Hz, H5′), 4.31 (dt, 1H, J = 6H, 2 Hz, H4′), 4.98 (dd, 1H, J = 6H, 2 Hz, H3′), 5.41 (dd, 1H, J = 6H, 2 Hz, H2′), 6.11 (br s, 2H, NH_2), 6.51 (d, 1H, J = 2 Hz, H1′), 8.36 (s, 1H, H2).

A mixture of compound **17** (21.15 g, 42.8 mmol), phenylboronic acid (20.9 g, 171.2 mmol), and $(Ph_3P)_4Pd$ (4.9 g, 4.3 mmol) was dissolved in 130 mL of diglyme and this was combined with 65 mL of 1 M aqueous Na_2CO_3. The resulting mixture was stirred for 4 h at 100 °C and then cooled and diluted with ether and water. The yellow solid that formed was filtered, and the layers were separated. The aqueous layer was extracted with ether. The combined ether extracts were washed with brine, dried ($MgSO_4$), and concentrated, which produced a white solid. The solid was collected by filtration and washed with ether to provide 12.7 g of compound **18d** contaminated by ca. 10 mol % of diglyme (ca. 58% yield). It was used without further purification: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 6H, Me_2Si), 0.82 (s, 9H, *t*-Bu), 1.40 (s, 3H, IP-CH_3), 1.62 (s, 3H, IP-CH_3), 3.67 (dd, 1H, J = 10, 6 Hz, H5′), 3.84 (dd, 1H, J = 10, 6 Hz, H5′), 4.34 (dt, 1H, J = 6, 2 Hz, H4′), 5.01 (dd, 1H, J = 6, 2 Hz, H3′), 5.52 (dd, 1H, J = 6, 2 Hz, H2′), 6.10 (br s, 2H, NH_2), 6.65 (d, 1H, J = 2 Hz, H1′), 7.5−7.6 (m, 3H, Ph), 7.6−7.8 (m, 2H, Ph), 8.46 (s, 1H, H2).

4-*N*-(Carbomethoxymethyl)amino-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (19a). General Method for Alkylation of an Amino Nucleoside with Methyl Bromoacetate. To a solution of compound **18a** (930 mg, 2.4 mmol) in 10 mL of THF at 0 °C was added a 1 M solution of potassium *tert*-butoxide in THF (2.7 mL, 2.7 mmol) and the mixture stirred for 5 min. Then methyl bromoacetate (0.25 mL, 2.7 mmol) was added and the mixture stirred for 1.5 h at room temperature. The mixture was concentrated by rotary evaporation; diluted with EtOAc; washed with 4 M NH_4Cl, water, and brine; dried ($MgSO_4$); and evaporated. The residue was subjected to chromatography on SiO_2 eluting with hexane/EtOAc mixtures of 8:1 and 5:1, which provided 399 mg (39%) of **19a** as a light yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, J = 7 Hz, 5′-CH_3), 1.31 and 1.53 (s each, 3H each, IP-CH_3s), 3.63 (s, 3H, ester CH_3), 4.15 (dq, 1H, J = 7, 4 Hz, H4′), 4.25 (d, 2H, J = 6 Hz, ester α-CH_2), 4.74 (dd, 1H, J = 7, 4 Hz, H3′), 5.34 (dd, 1H, J = 7, 3 Hz, H2′), 6.03 (t, 1H, J = 6 Hz, C4 NH), 6.25 (d, 1H, J = 3 Hz, H1′), 7.3−7.5 (m, 5H, Ar Hs), 7.57 (s, 1H, H6), 8.26 (s, 1H, H2).

4-*N*-(Carbomethoxymethyl)amino-5-phenyl-7-(*tert*-butyldimethylsilyloxyethoxymethyl)pyrrolo[2,3-*d*]pyrimidine (19b) was prepared as an amorphous solid from **18b** and methyl bromoacetate as described for **19a**. **19b** (23% yield): ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H, TBDMS−CH_3s), 0.86 (s, 9H, *t*-Bu), 3.59 (t, 2H, J = 5 Hz, OCH_2), 3.55 (s, 3H, ester CH_3), 3.75 (t, 2H, J = 5 Hz, OCH_2), 4.32 (d, 2H, J = 5 Hz, ester α-CH_2), 5.59 (t, 1H, J = 5 Hz, C4 NH), 5.68 (s, 2H, NCH_2O), 7.10 (s, 1H, H6), 7.3−7.6 (m, 5H, Ar Hs), 8.40 (s, 1H, H2).

4-*N*-(Carbomethoxymethyl)amino-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)-5-phenylpyrazolo[3,4-*d*]-pyrimidine (19c) was prepared as an amorphous solid from **18c** and methyl bromoacetate as described for **19a**. **19c** (53% yield): ^{1}H NMR (DMSO-d_6) δ 1.28 (d, 3H, J = 7 Hz, 5′-CH_3), 1.32 and 1.52 (s each, 3H each, IP-CH_3s), 3.64 (s, 3H, ester CH_3), 4.34 (dq, 1H, J = 7, 2 Hz, H4′), 4.28 (d, 2H, J = 6 Hz, ester α-CH_2), 4.81 (dd, 1H, J = 6, 2 Hz, H3′), 5.46 (dd, 1H, J = 6, 2 Hz, H2′), 6.39 (d, 1H, J = 2 Hz, H1′), 6.89 (t, 1H, J = 6 Hz, C4 NH), 7.5−7.8 (m, 5H, Ar Hs), 8.39 (s, 1H, H6).

7-(5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)-4-*N*-(carbomethoxymethyl)amino-5-phenylpyrazolo[3,4-*d*]pyrimidine (19d) was prepared as an amorphous solid from **18d** and methyl bromoacetate as described for **19a**. **19d** (ca. 40% yield): ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H, TBDMS−CH_3s), 0.82 (s, 9H, *t*-Bu), 1.40 (s, 3H, IP-CH_3), 1.61 (s, 3H, IP-CH_3), 3.62 (dd, 1H, J = 10, 6 Hz, H5′), 3.77 (dd, 1H, J = 10, 6 Hz, H5′), 3.78 (s, 3H, ester CH_3) 4.33 (dt, 1H, J = 6, 2 Hz, H4′), 4.35 (d, 2H, J = 5 Hz, ester α-CH_2), 5.02 (dd, 1H, J = 6, 2 Hz, H3′), 5.53 (dd, 1H, J = 6, 2 Hz, H2′), 6.00 (t, 1H, J = 5 Hz, C4 NH), 6.64 (d, 1H, J = 2 Hz, H1′), 7.5−7.8 (m, 5H, Ar Hs), 8.46 (s, 1H, H6).

4-*N*-(*N*-(*n*-Propyl)carbamoylmethyl)Amino-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-β-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (16f). General Method for $AlMe_3$-Mediated Preparation of Amides. To a solution of *n*-propylamine (0.20 mL, 2.4 mmol) in 5 mL of benzene at 0 °C was added a 2 M solution of $AlMe_3$ in THF (1.3 mL, 2.6 mmol) and the resulting mixture stirred at room temperature for 30 min, during which gas evolution occurred. The methyl ester **19a** (1.00 g, 2.36 mmol), as a solution in 5 mL of benzene, was cannulated into this solution. The resulting mixture was refluxed for 1 h and then cooled to 10 °C. Then 5 g of SiO_2 was added and the solvent evaporated. The resulting material was subjected to chromatography on SiO_2 eluting with hexane/EtOAc mixtures of 1:1 and 1:1.5, which provided 517 mg (49%) of compound **16f** as a white amorphous solid: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7 Hz, CH_3), 1.39 (s, 3H, IP-CH_3), 1.42 (d, 3H, J = 7 Hz, 5′-CH_3), 1.49 (sextet, 2H, J = 7 Hz, CH_2), 1.64 (s, 3H, IP-CH_3), 3.20 (q, 2H, J = 7 Hz, N−CH_2), 4.14 (d, 2H, J = 6 Hz, amide α-CH_2), 4.29 (dq, 1H, J = 7, 4 Hz, H4′), 4.69 (dd, 1H, J = 7, 4 Hz, H3′), 5.30 (dd, 1H, J = 7, 3 Hz, H2′), 5.59 (t, 1H, J = 7 Hz, C4 NH), 6.26 (d, 1H, J = 3 Hz, H1′), 6.49 (br s, 1H, C4 NH), 7.04 (s, 1H, H6), 7.4−7.5 (m, 5H, Ar Hs), 8.41 (s, 1H, H2).

This method was applied using an appropriate amine and methyl ester to prepare the precursors of the following compounds: **16g**−**j**, **26**, **28**, **32**−**41**, **43**−**46**, and **52**−**54**.

4-*N*-(*N*-Cyclopropylcarbamoylmethyl)amino-5-phenyl-7-(hydroxyethoxymethyl)pyrrolo[2,3-*d*]pyrimidine (16h). General Method for Desilylation. A mixture of 4-*N*-(*N*-cyclopropylcarbamoylmethyl)amino-5-phenyl-7-(*tert*-butyldimethylsilyloxyethoxymethyl)pyrrolo[2,3-*d*]pyrimidine (obtained from **19b**) (375 mg, 0.76 mmol) and 1 M tetrabutylammonium fluoride in THF (1.1 mL, 1.1 mmol) and 2.5 mL of CH_3CN was stirred at room temperature for 4 h. Then it was diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), and

concentrated to ca. 0.5 mL and diluted with ether to produce an oily suspension. The solvent was decanted from the residual oil. This was dissolved in benzene. Slow evaporation produced a waxy semisolid. The solvent was decanted and the residue dried at 75 °C/0.1 mm to produce a solid. This solid was triturated in hot water (80 °C), which on cooling produced 110 mg (38%) of compound **16h** as a white solid: mp 136−137 °C; ^{1}H NMR (DMSO-d_6) δ 1.3−1.45 (m, 2H, cy-Pr), 1.55−1.65 (m, 2H, cy-Pr), 2.5−2.7 (m, 1H, cy-Pr), 3.4−3.5 (m, 4H, −O(CH_2)$_2$O−), 4.03 (d, 2H, J = 5 Hz, CH_2C(O)), 4.68 (t, 1H, J = 6 Hz, OH), 5.61 (s, 2H, OCH_2N), 5.98 (t, 1H, J = 5 Hz, NH), 7.4−7.6 (m, 5H, Ph), 7.48 (s, 1H, H6), 8.15 (d, 1H, J = 4 Hz, NH), 8.26 (s, 1H, H2); UV (methanol) λ_{max} 282 nm (15 300). Anal. ($C_{20}H_{23}N_5O_3$) C, H, N.

4-*N*-(*N*-Cyclopropylthionocarbamoylmethyl)amino-5-phenyl-7-(5-deoxy-*β*-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (20). A mixture of compound **16b** (360 mg, 0.78 mmol) and Lawesson reagent (174 mg, 0.43 mmol) in 1 mL of THF was stirred for 72 h at room temperature and then more Lawesson reagent (79 mg, 0.2 mmol) was added and stirring continued at room temperature for 24 h. The solvent was evaporated, and the residue was suspended in CH_2Cl_2, adsorbed to SiO_2 by evaporation, and subjected to flash chromatography eluting with hexane/EtOAc mixtures of 2:1 and 1:1, which provided 204 mg (53%) of 4-*N*-(*N*-cyclopropylthionocarbamoylmethyl)amino-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 0.5−0.65 (m, 2H, cyclopropyl Hs), 0.7−0.8 (m, 2H, cyclopropyl Hs), 1.28 (d, 3H, J = 6 Hz, 5′-CH_3), 1.31 and 1.53 (s each, 3H each, IP-CH_3s), 2.5−2.7 (m, 1H, cyclopropyl H), 4.37 (d, 2H, J = 4 Hz, amide α-CH_2), 4.16 (dq, 1H, J = 6, 4 Hz, H4′), 4.75 (dd, 1H, J = 6, 4 Hz, H3′), 5.36 (dd, 1H, J = 4, 3 Hz, H2′), 6.25 (d, 1H, J = 3 Hz, H1′), 6.32 (m, 1H, C4 NH), 7.4−7.7 (m, 6H, Ar Hs and H6), 8.26 (s, 1H, H2), 10.07 (br s, 1H, amide NH). This compound was treated with 70% TFA in water as described for the preparation of compound **16c**. The residue obtained was recrystallized from ethanol/water to provide 129 mg (69%) of compound **20** as a crystalline solid: mp 97−100 °C; ^{1}H NMR (DMSO-d_6) δ 0.5−0.65 (m, 2H, cyclopropyl Hs), 0.7−0.8 (m, 2H, cyclopropyl Hs), 1.28 (d, 3H, J = 6 Hz, 5′-CH_3), 3.8−4.0 (m, 2H, H4′ and H3′), 4.36 (d, 2H, J = 5 Hz, amide α-CH_2), 4.47 (m, 1H, H-2′), 5.1 (br s, 1H, OH), 5.34 (d, 1H, J = 2 Hz, OH), 6.28 (t, 1H, J = 4 Hz, C4 NH), 6.12 (d, 1H, J = 5 Hz, H1′), 7.3−7.6 (m, 6H, Ar Hs and H6), 8.23 (s, 1H, H2), 10.07 (d, 1H, J = 2 Hz, amide NH). Anal. ($C_{22}H_{25}N_5O_3S\cdot0.75H_2O$) C, H, N.

4-*N*-(3-*N*-Methylureido)-5-phenyl-7-(5-deoxy-*β*-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (21). To a solution of compound **18a** (560 mg, 1.6 mmol) in 15 mL of THF were added methylisocyanate (0.12 mL, 2 mmol) and triethylamine (0.16 mL, 1.15 mmol). The resulting solution was refluxed for 17 h. The mixture was diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 eluting with 6:1 CH_2Cl_2/EtOAc to provide 500 mg (77%) of 4-*N*-(3-*N*-methylureido)-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine: ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, J = 7 Hz, 5′-CH_3), 1.31 and 1.54 (s each, 3H each, IP-CH_3s), 2.78 (d, 3H, J = 4 Hz, urea CH_3), 4.20 (dq, 1H, J = 6, 4 Hz, H4′), 4.76 (dd, 1H, J = 6, 4 Hz, H3′), 5.36 (dd, 1H, J = 6, 3 Hz, H2′), 6.31 (d, 1H, J = 3 Hz, H1′), 7.19 (s, 1H, NH), 7.4−7.6 (m, 6H, Ar Hs), 7.79 (s, 1H, H6), 8.54 (s, 1H, H2), 8.25 (d, 1H, J = 4 Hz, NH). This compound (500 mg, 1.2 mmol) was dissolved in 30 mL of methanol and 5 mL of 1 N aqueous HCl and the solution refluxed for 1 h. The residue was dissolved in water and saturated aqueous $NaHCO_3$ was added until the pH was 9. The product was filtered and washed with water and dried under vacuum at 75 °C to provide 350 mg (76%) of compound **21** as a solid: mp 201−202 °C; ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, J = 7 Hz, 5′-CH_3), 2.78 (d, 3H, J = 4 Hz, urea CH_3), 3.89−3.97 (m, 2H, H3′ and H4′), 4.53 (q, 1H, J = 5 Hz, H2′), 5.19 (d, 1H, J = 5 Hz, OH), 5.43 (d, 1H, J = 6 Hz, OH), 6.18 (d, 1H, J = 6 Hz, H1′), 7.16 (s, 1H, NH), 7.4−7.6 (m, 5H, Ar Hs), 7.76 (s, 1H, H6), 8.51 (s, 1H, H2), 9.29 (d, 1H, J = 4 Hz, NH). Anal. ($C_{19}H_{21}N_5O_4$) C, H, N.

4-*N*-(Propoxycarbonyl)amino-5-phenyl-7-(5-deoxy-*β*-d-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (22). To a solution of compound **18a** (1000 mg, 2.73 mmol) in 15 mL of CH_2Cl_2 and 15 mL of saturated aqueous $NaHCO_3$ was added propyl chloroformate (0.35 mL, 3.14 mmol) and the mixture stirred at room temperature for 16 h. The mixture was diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 eluting with 40:1 CH_2Cl_2:methanol to provide 558 mg (45%) of 4-*N*-(propoxycarbonyl)amino-5-phenyl-7-(5-deoxy-2,3-*O*-isopropylidenyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine: ^{1}H NMR (DMSO-d_6) δ 0.72 (t, 3H, J = 7 Hz, CH_3), 1.25 (quintet, 2H, J = 7 Hz, CH_2), 1.30 (d, 3H, J = 7 Hz, 5′-CH_3), 1.32 and 1.55 (s each, 3H each, IP-CH_3s), 4.13 (t, 1H, J = 7 Hz, OCH_2), 4.20 (dq, 1H, J = 6, 2 Hz, H4′), 4.77 (dd, 1H, J = 6, 4 Hz, H3′), 5.40 (dd, 1H, J = 6, 3 Hz, H2′), 6.35 (d, 1H, J = 3 Hz, H1′), 7.2−7.5 (m, 5H, Ar Hs), 7.88 (s, 1H, H6), 8.63 (s, 1H, H2), 10.15 (s, 1H, NH). This material was treated with 1 N aqueous HCl in methanol as described in the preparation of compound **21** to provide 275 mg (24%) of compound **22** as a solid: mp 98−99 °C; ^{1}H NMR (DMSO-d_6) δ 0.73 (t, 3H, J = 7 Hz, CH_3), 1.26 (quintet, 2H, J = 7 Hz, CH_2), 1.30 (d, 3H, J = 7 Hz, 5′-CH_3), 3.89−4.00 (m, 2H, H3′ and H4′), 4.57 (q, 1H, J = 5 Hz, H2′), 5.16 (d, 1H, J = 5 Hz, OH), 5.42 (d, 1H, J = 6 Hz, OH), 6.23 (d, 1H, J = 6 Hz, H1′), 7.2−7.5 (m, 5H, Ar Hs), 7.83 (s, 1H, H6), 8.61 (s, 1H, H2), 10.07 (s, 1H, NH); MS calcd for $C_{21}H_{24}N_4O_5$ M + 1 = 413, M + 1 found 413.

***N*-Cyclopropyl-*N*′-(5-phenyl-7-(5-deoxy-*β*-d-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-yl)oxalylbisamide (25). Step 1**. To a solution of oxalyl chloride monomethyl ester (0.50 mL, 5.4 mmol) in 5 mL of THF at room temperature was added triethylamine (0.76 mL, 5.4 mmol) and cyclopropylamine (0.38 mL, 5.4 mmol), and the mixture stirred for 3 h at room temperature. The resulting white solid was filtered and the filtrate evaporated to yield 546 mg (70%) of *N*-cyclopropyl-oxalylamide monomethyl ester (**23**), which was used without further purification.

Step 2. To a solution of compound **18a** (300 mg, 0.82 mmol) in 30 mL of THF at −10 °C was added 4.92 mL of 1 M potassium *tert*-butoxide (4.92 mmol) and the mixture stirred for 15 min. To this was added via cannula needle a solution of compound **23** (293 mg, 2.05 mmol) in 2 mL of THF. The resulting mixture was stirred at room temperature for 18 h and diluted with EtOAc and then water, the layers were separated, and the EtOAc layer was washed with water and brine, dried ($MgSO_4$), and evaporated to provide 265 mg of compound **24,** which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 0.5−0.7 (m, 4H, cyclopropyl Hs), 1.23 (d, 3H, J = 7 Hz, 5′-CH_3), 1.32 and 1.55 (s each, 3H each, IP-CH_3s), 2.6−2.8 (m, 1H, cyclopropyl H), 4.21 (dq, 1H, J = 7, 4 Hz, H4′), 4.79 (dd, 1H, J = 7, 4 Hz, H3′), 5.42 (dd, 1H, J = 7, 3 Hz, H2′), 6.36 (d, 1H, J = 3 Hz, H1′), 7.3−7.5 (m, 5H, Ar Hs), 7.96 (s, 1H, H6), 8.64 (d, 1H, J = 5 Hz, amide NH), 8.77 (s, 1H, H2), 10.73 (br s, 1H, amide NH).

Step 3. Compound **24** (265 mg) was combined with 3 mL of 70% aqueous TFA and stirred at room temperature for 2 h, and then saturated aqueous $NaHCO_3$ was slowly added until the mixture was neutral. An oil separated which became a solid after 6 h. The solid was collected by filtration, dried under high vacuum, and recrystallized twice from acetonitrile/water to provide 49 mg (14% from **18a**) of the title compound **25** as a solid: mp 150−153 °C; ^{1}H NMR (DMSO-d_6) δ 0.5−0.7 (m, 4H, cyclopropyl Hs), 1.31 (d, 3H, J = 7 Hz, 5′-CH_3), 2.6−2.8 (m, 1H, cyclopropyl H), 3.9−4.0 (m, 2H, H4′ and H3′), 4.60 (q, 1H, J = 6 Hz, H2′), 6.24 (d, 1H, J = 6 Hz, H1′), 7.3−7.5 (m, 5H, Ar Hs), 7.92 (s, 1H, H6), 8.63 (d, 1H, J = 5 Hz, amide NH), 8.74 (s, 1H, H2), 10.72 (s, 1H, amide NH). Anal. ($C_{22}H_{23}N_5O_3\cdot0.5H_2O$) C, H, N.

Water Solubility Determination. Into two vials, each containing 1 mg of test compound, was added 1 mL of DMSO and 1 mL of 100 mM MOPS pH 7.4 buffer, respectively. The vials were sonicated at room temperature for 15 min, let sit

for 10 min, and then filtered though 0.45 μm filters. The DMSO solution was diluted 10-fold with DMSO. A 50 μL sample of each solution was subjected to HPLC (UV detector set at 280 nm). Water solubility was calculated according to the equation:

$$\text{solubility} = ((\text{peak area}_{\text{buffer}})/(\text{peak area}_{\text{DMSO}} \times 10)) \times (1000\ \mu\text{g/mL}).$$

The water solubilities were single determinations, except for **16c**, which was performed twice to provide a solubility of 32 ± 9 μg/mL (mean ± SEM).

Human Recombinant Adenosine Kinase IC_{50} Determination. This was performed as previously described.[1a] Inhibitors were prescreened at concentrations of 10, 1, and 0.1 μM to determine the appropriate 11 different concentrations to use (in duplicate) for the IC_{50} determination. Control AK activity was determined under the assay conditions in the presence of 5% DMSO in triplicate. For each experiment performed, GP3269 served as a positive control at a concentration of 1 nM (in duplicate). A 1 nM GP3269 solution reduced recombinant human AK activity to 56 ± 1.6% of the control activity (mean ± SEM, 17 determinations in duplicate over 3 months). Under these assay conditions (three determinations in duplicate for each) the IC_{50}s ± SEM determined for GP3269 and 5-iodotubercidin were determined to be 1.5 ± 0.17[1b] and 26 ± 3.3 nM,[1a] respectively.

Rat Formalin Paw Pain Assay.[9] Fasted male SD rats (4 weeks, 105−115 g body weight) were used for each test. Three rats were used for each screening experiment and six rats were used for each dose−response group. The test compound was suspended in 0.1% methylcellulose (MC) and administered orally (20 mg/kg for screening and 0.3, 1, 3, and 10 mg/kg for ED_{50} determination; 0.5 mL/100 g rat) 15 min before the formalin challenge. Animals were habituated to the observation chamber for 10 min and then 50 μL of 2% formalin solution was injected subcutaneously into the plantar surface of the right hindpaw. They were monitored for pain related behaviors[9] at 0−5 min (1st phase) and 15−30 min (2nd phase) after the challenge. Licking and/or biting behavior was scored for 5 s in every 30 s as follows: 0 = no licking and/or biting; 1 = licking and/or biting behaviors observed. Inhibition values are reported for the 2nd phase. The intra-assay coefficient of variation in the 2nd phase control licking/biting response on treatment with vehicle averaged 20.70% after monitoring once weekly for 18 weeks (n = 5/week). The range of the intra-assay coefficient of variation for this period was 6−41%. The inter-assay coefficient of variation for the control was 9.00%. For each experiment performed, 5-phenyl-4-*N*-phenylamino-7-α-L-lyxofuranosylpyrrolo-[2,3-*d*]pyrimidine (GP790)[1d,3a,6a] served as a positive control (5 mg/kg po dosing) and averaged 87 ± 9% (mean ± SEM, 12 determinations, n = 3 each) inhibition of the licking/biting response in the 2nd phase.

Hepatotoxicity Assay. Test compound was dissolved in 75:25 PEG-400/saline and administered intravenously to three male rats, a total of 75 μmols/kg for each, over two 40-min infusions, 12 h apart. A blood sample was taken 24 h after the first infusion, and serum chemistry levels of the following liver enzymes were determined at Pathology Medical Laboratories, San Diego, CA: T bilirubin, SGOT, SGPT, creatine, GGT, albumin, GLU, BUN, ALP.

Rat Rotorod Assay. The test compound was orally administered at 10−300 mg/kg doses in 0.1% MC to fasted male SD rats (n = 6 for each dose response group, 94−104 g body weight). CNS side effects, sedation and ataxia, were determined by rotorod treadmill (Natsume Seisakusho) rotated at 5 rpm. Rats were trained to walk on the rod for 150 s. The walking time was measured at 0, 0.5, 1, 2, and 5 h after administration with a cutoff time at 150 s.

Rat Carrageenan-Induced Mechanical Hyperalgesic Test.[28] Fasted male SD rats (94−118 g body weight) were used for each test. The test compound was suspended in 0.1% MC and administered orally (0.5 mL/100 g rat) at 1, 3, and 10 mg/kg 1 h before (for prophylactic test) or 2 h after (for therapeutic test) carrageenan injection (1%, 100 μL/paw i.pl.). Analgesic tests were performed at 3, 4, 5, and 6 h after carrageenan injection using the Randall−Selitto analgesimeter.

Pharmacokinetics in Rat and Dog. Solutions of test compound, at 3 mg/kg in 0.1% MC, were administered to rats or dogs by po or iv routes, plasma samples were taken at 0.25, 0.5, 1, 2, 4, 8, and 24 h, and test compound concentrations were determined by HPLC.

Stability in Human Liver Microsomes. The test compound was incubated at a concentration of 1 μM with human liver microsomes (1.5 mg/mL), and samples were removed and evaluated by HPLC for the percent test compound remaining at 0, 0.5, 1, 2, and 4 h after initiation of the incubation.

Bradykinin-Induced Licking Assay on Marmoset Monkeys.[29] Four to six individually housed male marmoset monkeys (*Callithrix jacchus*) weighing 293−359 g were each treated with the test compound at doses of 0.1, 1.0, and 10 mg/ kg po or with vehicle, 0.1% MC (0.5 mL/100 g body weight po) for the control. The appearance and behavior of the monkeys were monitored and videotaped individually for 75 min following test compound administration. At 60 min after the administration, 100 μL of a 1 mM bradykinin solution in physiological saline was i.pl. injected into the foot pad of the animals. The licking, scratching, and biting in response to the bradykinin administration was recorded on videotape for 15 min and the results were analyzed to give the pain score. The scores are as follows: 0 = moving the injected leg normally; 1 = keeping the injected leg attached to the body; 2 = scratching or licking of the injected area of the leg, and 3 = intensively and repetitively scratching or licking the injected area of the leg. Every 15 s a score was made over a period of 15 min as an index of pain behavior. Ataxia, sedation, and emesis were also monitored. The animals were subjected to the experiments with an interval of 2 weeks between experiments and bradykinin was injected into right and left foot pads alternately.

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Supporting Information Available: Melting points, microanalysis, and ^{1}H NMR data for all compounds evaluated for adenosine kinase inhibition. Dose−response graphs for the rat formalin paw assay, rat mechanical hyperalgesic assay, rat rotorod assay, monkey bradykinin-induced licking assay, full PK data in rats and dogs, and liver panel data for compound **16c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Ugarkar, B. G.; DaRe, J. M.; Kopcho, J. J.; Browne, C. E. III; Schanzer, J. M.; Wiesner, J. B.; Erion, M. D. Adenosine Kinase Inhibitors. 1. Synthesis, Enzyme Inhibition and Antiseizure Activity of 5-Iodotubercidin Analogues. *J. Med. Chem.* **2000**, *43*, 2883−2893. (b) Ugarkar, B. G.; Castellino, A. J.; DaRe, J. M.; Kopcho, J. J.; Wiesner, J. B.; Schanzer, J. M.; Erion, M. D. Adenosine Kinase Inhibitors. 2. Synthesis, Enzyme Inhibition and Antiseizure Activity of Diaryltubercidin Analogues. *J. Med. Chem.* **2000**, *43*, 2894−2905. (c) Erion, M. D.; Ugarkar, B. G.; DaRe, J.; Castellino, A. J.; Fujitaki, J. M.; Dixon, R.; Appleman, J. R.; Wiesner, J. B. Design, Synthesis and Anticonvulsant Activity of the Potent Adenosine Kinase Inhibitor GP2639. *Nucleosides, Nucleotides Nucleic Acids* **1997**, *16*, 1013−1021. (d) Weisner, J. B.; Ugarkar, B. G.; Castellino, A. J.; Barankiewicz, J.; Dumas, D. P.; Gruber, H. E.; Foster, A. C.; Erion, M. D. Adenosine Kinase Inhibitors as a Novel Approach to Anticonvulsant Therapy. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1669−1677. (e) Pak, M. A.; Hass, H. L.; Decking, U. K. M.; Schrader, J. Inhibition of Adenosine Kinase Increases Endogenous Adenosine and Depresses Neuronal Activity in Hippocampal Slices. *Neuropharmacology* **1994**, *33*, 1049−1053.

(2) Tatlisumak, T.; Takano, K.; Carano, R.; Miller, L. P.; Foster, A. C.; Fisher, M. Delayed Treatment with an Adenosine Kinase Inhibitor, GP683, Attenuates Infarct Size in Rats with Temporary Middle Cerebral Artery Occlusion. *Stroke* **1998**, *29*, 1952−1958.

(3) (a) Ugarkar, B. G.; Castellino, A. J.; DaRe, J. S.; Ramirez-Weinhouse, M.; Kopcho, J. J.; Rosengren, S.; Erion, M. D. Adenosine Kinase Inhibitors. 3. Synthesis, SAR and Antiinflammatory Activity of a Series of L-Lyxofuranosyl Nucleosides. *J. Med. Chem.* **2003**, *46*, 4750−4760. (b) Firestein, G. S.; Boyle, D.; Bullough, D. A.; Gruber, H. E.; Sajjadi, F. G.; Montag, A.; Sambol, B.; Mullane, K. M. Protective effect of an adenosine kinase inhibitor in septic shock. *J. Immunol.* **1994**, *152*, 5853−5859. (c) Firestein, G. S.; Bullough, D. A.; Erion, M. D.; Jimenez, R.; Ramirez-Weinhouse, M.; Barankiewicz, J.; Smith, C. W.; Gruber, H. E.; Mullane, K. M. Inhibition of neutrophil adhesion by adenosine and an adenosine kinase inhibitor: The role of selectins. *J. Immunol.* **1995**, *154*, 326−334.

(4) Villarreal, F.; Zimmermann, S.; Makhsudova, L.; Montag, A. C.; Erion, M. D.; Bullough, D. A.; Ito, B. R. Modulation of cardiac remodeling by adenosine: In vitro and in vivo effects. *Mol. Cell. Biochem.* **2003**, *251*, 17−26.

(5) (a) Keil II, G. J.; DeLander, G. E. Adenosine kinase and adenosine deaminase inhibition modulate spinal adenosine- and opiod agonist-induced antinociception in mice. *Eur. J. Pharmacol.* **1994**, *271*, 37−46. (b) Sawynok, J.; Liu, X. J. Adenosine in the spinal cord and periphery: Release and regulation of pain. *Prog. Neurobiol.* **2003**, *69*, 313−340.

(6) (a) Boyer, S. H.; Ugarkar, B. G.; Solbach, J.; Kopcho, J.; Matelich, M. C.; Ollis, K.; Gomez-Galeno, J. E.; Mendonca, R.; Tsuchiya, M.; Nagahisa, A.; Nakane, M.; Wiesner, J. B.; Erion, M. D. Adenosine Kinase Inhibitors. 5. Synthesis, Enzyme Inhibition and Analgesic Activity of Erythro Diarylturbercidin Analogs. *J. Med. Chem.* **2005**, *48*, 6430−6441. (b) Preliminary report: Ugarkar, B. G.; Lemus, R. H.; Gomez-Galeno J. E.; Matelich, M. C.; Boyer, S. H.; Wiesner, J. B.; Booten, S.; Erion, M. D.; Tsuchiya, M.; Nakane, M.; Nagahisa, A. Adenosine Kinase Inhibitors 1. A New Class of Potential Analgesic Agents. *Abstr. Pap.—Am. Chem. Soc*. **2001**, *221*, CARB-032. (c) Jarvis, M. F.; Yu, H. X.; Kohlhaas, K.; Alexander, K.; Lee, C. H.; Jiang, M. Q.; Bhagwat, S. S.; Williams, M.; Kowaluk, E. A. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine, a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties: I. In vitro characterization and acute antinociceptive effects in the mouse. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1156−1164. (d) Kowaluk, E. A.; Mikusa, J.; Wismer, C. T.; Zhu, C. Z.; Schweitzer, E.; Lynch, J. J.; Lee, C. H.; Jiang, M. Q.; Bhagwat, S. S.; Gomtsyan, A.; McKie, J.; Cox, B. F.; Polakowski, J.; Reinhart, G.; Williams, M.; Jarvis, M. F. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine, a novel orally effective adenosine kinase inhibitor with analgesic and antiinflammatory properties: I. In vivo characterization in the rat. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1165−1174.

(7) (a) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine Receptors: Pharmacology, Structure−Activity Relationships, and Therapeutic Potential. *J. Med. Chem.* **1992**, *35*, 407−422. (b) Erion, M. D. Adenosine Receptors as Pharmacological Targets. *Annu. Rep. Med. Chem.* **1993**, *28*, 295−304. (c) *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997. (d) Williams, M.; Jarvis, M. F. Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem. Pharmacol.* **2000**, *59*, 1173−1185.

(8) (a) Bookser, B. C.; Matelich, M. C.; Ollis, K.; Ugarkar, B. G. Adenosine Kinase Inhibitors. 4. 6,8-Disubstituted Purine Nucleoside Derivatives. Synthesis, Conformation, and Enzyme Inhibition. *J. Med. Chem.* **2005**, *48*, 3389−3399. (b) Another approach to ARAs is by inhibition of AMP deaminase. See for example: Erion, M. D.; Kasibhatla, S. R.; Bookser, B. C.; van Poelje, P. D.; Reddy, M. R.; Gruber, H. E.; Appleman, J. R. Discovery of AMP Mimetics that Exhibit High Inhibitory Potency and Specificity for AMP Deaminase. *J. Am. Chem. Soc.* **1999**, *121*, 308−319.

(9) Dubuisson, D.; Dennis, S. G. The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brainstem stimulation in rats and cats. *Pain* **1977**, *4*, 161−164.

(10) Woolfe, G.; MacDonald, A. D. The evaluation of the analgesic action of pethidine hydrochloride (demeral) *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300−307.

(11) D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74−79.

(12) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−23.

(13) (a) Curatolo, W. Physical chemical properties of oral drug candidates in the discovery and exploratory development settings. *Pharm. Sci. Technol. Today* **1998**, *1*, 387−393. (b) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235−249.

(14) (a) Gewald, K. Reaktion von Malonsauredinitril mit α-Aminoketonen. *Z. Chem.* **1961**, *1*, 349. (b) Abdalla, G. M.; Sowell, J. W. Sr. Synthesis of 8-Cyano-1,4-dihydro-4-oxopyrrolo[1,2-*a*]pyrimidine-3-carboxylic Acids as Potential Antimicrobial Agents. *J. Heterocycl. Chem.* **1987**, *24*, 297−301.

(15) Wilcox, C. S.; Otoski, R. M. Stereoselective Preparations of Ribofuranosyl Chlorides and Acetates. Solvent Effects and Stereoselectivity in the Reaction of Ribofuranosyl Acetates with Trimethylallylsilane. *Tetrahedron Lett.* **1986**, *27*, 1011−1014.

(16) Cohen, Noal; Banner, B. L.; Lopresti, R. J.; Wong, F.; Rosenberger, M.; Liu, Y.-Y.; Thom, E.; Liebman, A. A. Enantiospecific Synthesis of Leukotrienes C_4, D_4, and E_4 and [14,15-$^3H_2$]-Leukotriene E_4 Dimethyl Ester. *J. Am. Chem. Soc.* **1983**, *105*, 3661−3672.

(17) Use of the Vilsmeyer salt to prepare chloro sugars will be reported in detail elsewhere: Kopcho, J.; Ugarkar, B. G. Unpublished research.

(18) Wang, G.; Ta, R. C.; Gunic, E.; Du, J.; Bard, J.; Pai, B. Synthesis and Cytokine Modulation Properties of Pyrrolo[2,3-*d*]-4-pyrimidone Nucleosides. *J. Med. Chem.* **2000**, *43*, 2566−2574.

(19) The synthesis of amino acid substituted nucleosides has precedent going back to the pioneering work of Carter and others: (a) Carter, C. E. Synthesis of 6-Succinioaminopurine. *J. Biol. Chem.* **1956**, *223*, 139−146. (b) Ward, D. N.; Wade, J.; Walborg, E. F. Jr.; Osdene, T. S. The Synthesis of *N*-(6-Purinyl)amino Acids. Amino Acids with a Single Reactive Amino Group. *J. Org. Chem.* **1961**, *26*, 5000−5005. (c) Vickers, R. S.; Gerster, J. F.; Robins, R. K. *N*-(2-Amino-9-*β*-D-ribofuranosyl-9*H*-purin-6-yl)-glycine. Preparation of a Nucleoside Derivative of an Amino Acid in *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W.; Tipson, R. S.; Eds.; John Wiley and Sons: New York, 1968; pp. 281−282, Chapter 90.

(20) (a) Taylor, E. C.; Hendess, R. W. Synthesis of Pyrrolo[2,3-*d*]-pyrimidines. The Aglycon of Toyocamycin. *J. Am. Chem. Soc.* **1965**, *87*, 1995−2003. (b) Widler, L.; Green, J.; Missbach, M.; Susa, M.; Altmann, Eva 7-Alkyl- and 7-Cycloalkyl-5-aryl-pyrrolo-[2,3-*d*]pyrimidines—Potent Inhibitors of the Tyrosine Kinase c-Src. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 849−852.

(21) Cottam, H. B.; Petrie, C. R.; McKernan, P. A.; Goebel, R. J.; Dalley, N. K.; Davidson, R. B.; Robins, R. K.; Revankar, G. R. Synthesis and Biological Activity of Certain 3,4-Disubstituted Pyrazolo[3,4-*d*]pyrimidine Nucleosides. *J. Med. Chem.* **1984**, *27*, 1119−1127.

(22) (a) Kobayashi, S. The Synthesis and Xanthine Oxidase Inhibitory Activity of Pyrazolo[3,4-*d*]pyrimidines. *Chem. Pharm. Bull.* **1973**, *21*, 941−951. (b) Tominaga, Y,; Matsuoka, Y.; Oniyama, Y.; Uchimura, Y.; Komiya, H.; Hirayama, M.; Kohra, S.; Hosomi, A. Polarized Ethylenes. IV [1]. Synthesis of Polarized Ethylenes Using Thioamides and Methyl Dithiocarboxylates and Their Application to the Syntheses of Pyrazoles, Pyrimidines, Pyrazolo-[3,4-*d*]pyrimidines and 5-Aza[2.2.3]cyclazines. *J. Heterocycl. Chem.* **1990**, *27*, 647−660. (c) Kraybill, B. C.; Elkin, L. L.; Blethrow, J. D.; Morgan, D. O.; Shokat, K. M. Inhibitor Scaffolds as New Allele Specific Kinase Substrates. *J. Am. Chem. Soc.* **2002**, *124*, 12118−12128.

(23) Browne, C. E.; Ugarkar, B. G.; Mullane, K. M.; Gruber, H. E.; Bullough, D. A.; Erion, M. D.; Castellino, A. J. Adenosine Kinase Inhibitors. U.S. Patent No. 5,864,033, January 1999.

(24) (a) Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev.* **1995**, *95*, 2457−2483. (b) Suzuki, A. In *Metal-Catalyzed Cross-Coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, Germany, 1998; Chapter 2.

(25) (a) Basha, A.; Lipton, M.; Weinreb, S. M. A Mild, General Method for Conversion of Esters to Amides. *Tetrahedron Lett.* **1977**, 4171−4174. (b) Levin, J. I.; Turos, E.; Weinreb, S. M. An Alternative Procedure for the Aluminum-Mediated Conversion of Esters to Amides. *Synth. Commun.* **1982**, *12*, 989−993. (c) Lipton, M. F.; Basha, A.; Weinreb, S. M. Conversion of Esters to Amides with Dimethylaluminum Amides: *N*,*N*-Dimethylcyclohexanecarboxamide. *Org. Synth.* **1979**, *59*, 49−53.

(26) (a) Scheibye, S.; Pedersen, B. S.; Lawesson, S.-O. Studies on Organophosphorous Compounds XXI. The Dimer of 2-Methoxyphenylthionophosphine Sulfide as Thiation Reagent. A New Route to Thiocarboxamides. *Bull. Soc. Chim. Belg.* **1978**, *87*, 229−238. (b) For a review of the Lawesson's reagent see Cava, M. P.; Levinson, M. I. Thionation Reactions of Lawesson's Reagents. *Tetrahedron* **1985**, *41*, 5061−5087.

(27) Additional modified sugar and pyrazolopyrimidine diaryltubercidine analogues: Ugarkar, B. G.; Matelich, M. C.; Boyer, S. Unpublished research.

(28) Randal, L. O.; Selito, J. J. A method for measurement of analgesic activity on inflamed tissue. *Arch. Int. Pharmacodyn.* **1957**, *111*, 409−419.

(29) The use of bradykinin is a modification of a known procedure employing formalin: Alreja, M.; Mutalik, P.; Mayar, U.; Manchanda, S. K. The formalin test: A tonic pain model in the primate. *Pain* **1984**, *20*, 97−105.

(30) (a) Mathews, I. I.; Erion, M. D.; Ealick, S. E. Structure of Human Adenosine Kinase at 1.5 Å Resolution. *Biochemistry* **1998**, *37*, 15607−15620. (b) Schumacher, M. A.; Scott, D. M.; Mathews, I. I.; Ealick, S. E.; Roos, D. S.; Ullman, B.; Brennan, R. G. Crystal Structures of *Toxoplasma gondii* Adenosine Kinase Reveal a Novel Catalytic Mechanism and Prodrug Binding. *J. Mol. Biol.* **2000**, *296*, 549−567. (c) Cook, W. J.; DeLucas, L. J.; Chattopadhyay, D. Crystal structure of adenosine kinase from *Toxoplasma gondii* at 1.8 Å resolution. *Protein Sci.* **2000**, *9*, 704−712.

(31) (a) Ojima, H.; Yamada, K. Synthesis and Characterization of μ-bis[*N*-(hydroxypropyl)glycinamidato(2−)]dicopper(II) and μ-bis-[*N*-(hydroxypropyl)-DL-alaninamidato(2−)]dicopper(II). *Z. Anorg. Allg. Chem.* **1970**, *379*, 322−8. (b) Angrick, M.; Rewicki, D. Reactions of Sugars and Related Polyfunctional Compounds with Amino Acids. 2. N-Acylation of 3-Amino-1,2-propanediol with Amino Acids. *Liebigs Ann. Chem.* **1982**, 1394−1397.

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