Adenosine Kinase Inhibitors. 4. 6,8-Disubstituted Purine Nucleoside Derivatives. Synthesis, Conformation, and Enzyme Inhibition

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Received December 21, 2004

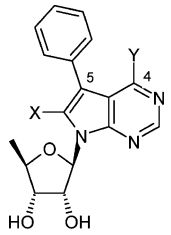
6,8-Disubstituted purine nucleosides were synthesized and evaluated as adenosine kinase inhibitors (AKIs). A method was developed to selectively substitute arylamines for halogens at C6 and C8 which utilizes alkali salts of arylamino anions. Regioselectivity was found to be counterion dependent. Potassium and sodium salts add selectively to C6 of 6-chloro-8-iodo-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (**7a**) while lithium salts add to C6 and C8 positions. Differential 6,8-bisarylamin-*N,N*′-diylpurine nucleosides such as 8-anilin-*N*-yl-6-indolin-*N*-yl-9-(β-D-ribofuranosyl)purine (**10b**) can be prepared by employing stepwise reactions of potassium and then lithium salts of different arylamino anions followed by fluoride ion-induced desilylation. Other C8-substituted compounds were prepared by way of either C8 lithiation chemistry or palladium cross-coupling reactions. Several of these compounds were potent AKIs (e.g. **10b**, AK $IC_{50} = 0.019$ μM) and are more potent than the previous best purine-based AKI 5′-deoxy-5′-aminoadenosine (AK $IC_{50} = 0.170$ μM). AK inhibitory potency was greatest for those compounds with ^{1}H NMR evidence of a predominant anti glycosyl bond conformation, whereas most analogues adopt a syn conformation because of steric repulsions between the C8 substituent and the ribose group. The inhibitors are proposed to bind in the anti conformation with the hydrophobic C6 and C8 substituents contributing to AK affinity in a manner similar to the C4 and C5 aryl substituents of the potent diaryltubercidin nucleoside inhibitor series.

Introduction

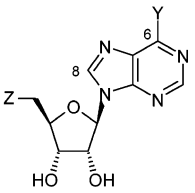
Adenosine kinase inhibitors (AKIs) have demonstrated therapeutic potential in a variety of animal models of epilepsy,[1] stroke,[2] inflammation,[3] acute myocardial infarction,[4] and analgesia.[5] Therapeutic efficacy results from increased levels of extracellular adenosine activating A_1 and/or A_2 adenosine receptors in a site- and event-specific manner. Because AKIs can be used to generate localized increases in adenosine concentration, they are part of a group classified as adenosine regulating agents (ARAs).[6,7] While considerable work has been performed to optimize pyrrolopyrimidine and pyrazolopyrimidine nucleoside AKIs,[1−4] other structure classes are desirable to overcome issues such as poor oral activity, weak efficacy, and/or toxicity.

One structural class that has received limited exploration for AKIs is purine nucleosides. Table 1 shows a brief comparison of pyrrolopyrimidine- and purine-based nucleoside AK structure−activity relationships (SARs). Prior work in the pyrrolopyrimidine series of AKIs revealed SARs that depended on the substitution of aryl groups at the C4 and C5 positions of the base to produce potent AKIs. Compound **1** with AK $IC_{50} = 0.0005$ μM is the prototypic member of this class designated as diaryltubercidins.[1b] In the purine series, an analogue of similar potency has not yet been discovered, and the most potent AKI known in this class is 5′-amino-5′-deoxyadenosine (**4**, AK $IC_{50} = 0.17$ μM).[1a,8,9] To access the same binding interactions and thus similar potencies as the diaryltubercidin-based AKIs, substitution of hydrophobic groups at positions C6 and C8 was considered. A previously discovered C6-substituted purine nucleoside, the indolinyl-substituted analogue **5** (AK $IC_{50} = 0.50$ μM),[10] provided a lead from which to explore C8-substituted analogues. N^6-Phenyladenosine has also previously been evaluated as a substrate and inhibitor of AK ($K_i = 0.8$ μM and $K_m = 2$ μM).[9] So aniline and indoline are reasonable substituents to explore at C6. It is interesting to note that in compound **3**, C4 indoline substitution in the diaryltubercidin series resulted in a 100-fold weaker inhibitor compared to aniline-substituted compound **1** (Table 1). Evidence that C8 substitution could be tolerated as part of an AKI structure may be inferred from the 6-bromodiaryltubercidin analogue

Table 1. Early Comparative SAR



1-3



4-5

compd	X	Y	Z	AK IC_{50} (μM)
1[a]	H	anilin-*N*-yl	−	0.0005
2[a]	Br	anilin-*N*-yl	−	0.001
3[b]	H	indolin-*N*-yl	−	0.051
4[c]	−	NH_2	NH_2	0.17
5[d]	−	indolin-*N*-yl	OH	0.50

[a] See ref 1b. [b] See Experimental Section. [c] See ref 1a. [d] See ref 10.

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10.1021/jm048968j CCC: $30.25
Published on Web 04/07/2005

2 which did not exhibit a significant loss in inhibitory potency compared to **1** (AK $IC_{50} = 0.001\ \mu M$).[1b]

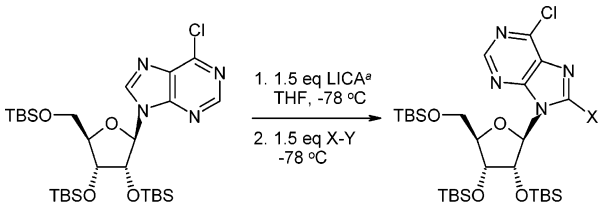
To optimize the purine series, one issue to consider was the known preference for a syn orientation about the glycosyl bond with C8-substituted purine nucleosides. C8-Unsubstituted purine nucleosides prefer an anti conformation.[11,12] Early nucleoside conformational studies demonstrated, using circular dichroism[13] and NMR spectroscopy,[13,14] that in aqueous solution, purine nucleosides with bromo, fluoro, methylthio, methyl, and other small alkyl group substitutents at C8 were predominantly syn. The syn conformation is lower in energy for these C8-substituted nucleosides because steric repulsions between the 8-substituent and the ribose ring make the anti conformation less favorable. This is an important consideration since X-ray crystal structures of human and parasitic AK show that substrates and inhibitors bind in an anti conformation.[15] However, further NMR experiments have confirmed that the rapid syn−anti equilibration of C8-substituted nucleosides affords access to the anti conformer in solution.[16] This access has been demonstrated to allow enzyme binding of C8-substituted nucleosides and nucleotides in their anti conformations. For example, the nucleotides 8-bromoadenosine diphosphoribose and the 8-bromo analogue of NAD^+, which prefer a syn conformation in solution, adopt an anti conformation when bound to the active site of certain dehydrogenase enzymes as demonstrated by X-ray crystallography.[17] Furthermore, and relevant to the current study, the syn nucleoside 8-bromoadenosine[13] was found to be a substrate for AK ($K_m = 11\ \mu M$).[9] Thus it was reasonable to expect that C8-substituted purine nucleosides could bind to AK in an anti conformation due to rapid syn−anti equilibration.

To optimize the purine nucleoside **5** for inhibition of AK, C8 substituents of varying size and hydrophobicity were explored. It was unknown what C8 substitutuent would enable optimal binding to AK. It was anticipated the C8 substituent would provide increased affinity by binding to the same hydrophobic region thought to bind the C5 phenyl group of the diaryltubercidin inhibitors (e.g. **1**). A variety of 6,8-disubstituted purine nucleosides were envisioned to be accessible from the known 6-chloro-8-iodopurine nucleoside intermediate **7a**.[18] Synthesis of 8-arylaminopurine nucleosides represented a special synthetic challenge, yet the nucleosides were highly desired because of their potential hydrophobic similarity to diaryltubercidins. While thermal addition (80−118 °C) of arylamines to displace C6-chloro substitutents on purines is readily accomplished,[10] similar reactions to displace C8-halogens have not been reported. In a separate study, we found that under conditions where alkylamines substitute at C8 of 8-bromoadenosine (130 °C, DMSO),[19] aniline was unreactive and increased temperatures resulted only in decomposition of the nucleoside. Thus milder reaction conditions were necessary for the preparation of this analogue type.

Several alternative methods have been reported for the synthesis of 8-arylaminopurine nucleosides. Recently, 8-bromopurine intermediates have been reported to undergo cross-coupling with arylamines using specialized palladium-catalyzed reaction conditions.[20] Also, 8-anilin-*N*-yladenosine has been prepared in low yield by reaction of adenosine with *N*-benzoyloxy-*N*-phenyl hydroxylamine.[21] Both of these methods require handling of air-sensitive reagents. A common route to 8-arylamino-substituted purine bases requires cyclization of 5,6-diaminopyrimidines.[22] However, this method would require the additional step of ribosylation of the 8-substituted base to complete the nucleoside synthesis.

We sought to discover an alternative method of C8-arylamino substitution that was mild and practical to apply. In this report we describe novel synthetic methodology where lithium anilinylamide addition to C8-iodopurine nucleosides is applied to produce C8-arylamino-substituted analogues of **5**. Consistent with our hypothesis that 6,8-disubstituted purine nucleosides can be potent AK inhibitors, a few of these compounds were found to be sub-100 nM inhibitors of the enzyme.

Table 2. C8 Anion Chemistry



6 **7**

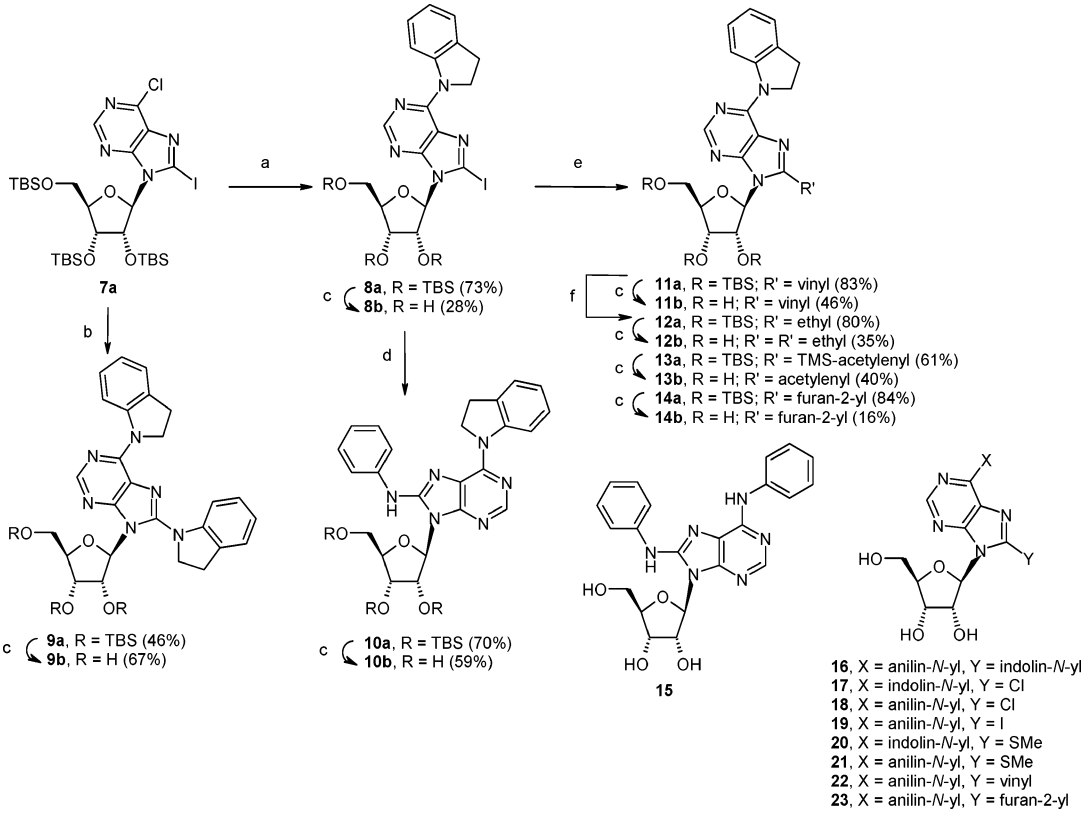
compd	reagent X−Y	X	% yield
7a[b]	I_2	I	100
7b	CCl_3CCl_3	Cl	86
7c	$MeS-SO_2Me$	SMe	79

[a] LICA = lithium isopropylcyclohexylamide. [b] See also ref 18.

Chemistry. Access to differentially 6,8-disubstituted purine nucleosides was achieved in three steps starting from 6-chloro-8-iodopurine riboside intermediate **7a**.[18] Compound **7a** is readily obtained in two steps from commercially available 6-chloro-9-(β-D-ribofuranosyl)-purine.[23] The key step involves deprotonation of the tris-*tert*-butyldimethylsilyl protected nucleoside **6** with lithium isopropylcyclohexylamide (LICA) followed by reaction of the C8 lithio intermediate with iodine to form **7a** in high yield. This lithiation approach to C8-derivatization was also used to prepare 8-chloro (**7b**) and 8-methylthio (**7c**) analogues in high yields (see Table 2).[24]

Utilizing 6,8-dihalonucleoside **7a** as the substrate, it was recognized that while thermal methods could be used for C6 arylamine addition, C8 arylamine addition had little precedent[20] and would require development of new methodology. A low-temperature amino anion addition reaction was studied as a means of introducing C8 arylamino groups.[25] Arylamino anions substituted at C6 of **7a**, but efficient C8 substitution depended on the salt form (lithium, sodium, or potassium) of the arylamino anion used for the addition reaction (Scheme 1).[24] The potassium salt of indolin-*N*-yl anion (2 equiv), formed using potassium *tert*-butoxide (2 equiv) in THF at −20 °C, reacted selectively at C6 of **7a** to produce **8a** (73%) with less than 5% of the bis-substituted derivative **9a** formed. The sodium salt (NaH/THF) also selectively produced **8a** but in variable yield (37−62%). However, use of the indolin-*N*-yl anion lithium salt, formed with *n*-BuLi and reacted under the same conditions, produced bis-substituted **9a** (46%) with no recovery of the mono

Scheme 1. Selective Substitution of C6 vs C8[a]



[a] (a) 2 equiv of indoline, 2 equiv of *t*-BuO$^-$K$^+$, THF, −20 °C; (b) 2 equiv of indoline, 2 equiv of *n*-BuLi, THF, −20 °C; (c) 6 equiv of $Et_4N^+F^-$, 4 equiv of AcOH, DMF; (d) 3 equiv of aniline, 3 equiv of *n*-BuLi, THF, −20 °C to room temperature; (e) for **11a**: tributyl(vinyl)tin, $Pd(PPh_3)_4$, CuO, DMF; for **13a**: (trimethylsilyl)acetylene, $(Ph_3P)_2PdCl_2$, CuI, NEt_3; for **14a**: 2-(tributylstannyl)furan, $Pd(PPh_3)_4$, DMF; (f) H_2, Pd/C.

adduct **8a** or reactant **7a**. Displacement of both halogens on **7a** by lithium anilinylamide required higher temperatures and excess reagents (6 equiv of aniline, 6 equiv of *n*-BuLi, −20 to 22 °C, 16 h) to produce the tris-TBS derivative of **15** (74%). One example where this selectivity was exploited to produce differentially 6,8-disubstituted purine nucleoside analogues is the two-step synthesis of **10a** (Scheme 1). Thus, by conducting two similar reactions on **7a**, the first with an arylamino anion potassium salt and the second with a lithium salt of a different arylamino anion, diverse 6,8-bis-arylamino-substituted purine nucleosides were prepared.

Additional C8-substituted analogues were prepared from **8a** by applying Stille and Sonogashira palladium-catalyzed cross-coupling reactions (Scheme 1). Methods previously established on 8-bromo- and 8-iodopurine nucleosides[26] were used to prepare C8 vinyl, acetylenyl, and furan-2-yl derivatives (**11a, 13a**, and **14a**, respectively). The vinyl derivative **11a** was hydrogenated to the ethyl compound **12a**.

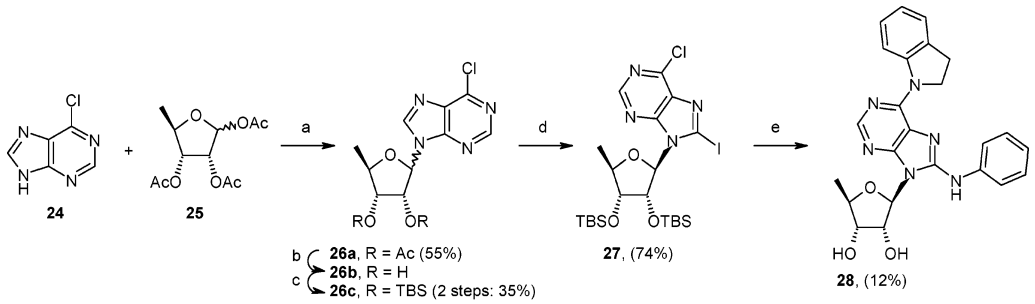
Removal of the *tert*-butyldimethylsilyloxy protecting groups was performed with tetraethylammonium fluoride (6 equiv) in DMF buffered by acetic acid (4 equiv) to produce nucleosides **8b**, **9b**, **10b**, **11b**, **12b**, **13b**, **14b**, **17**, and **20** in variable yields (16−67%, see Scheme 1). In the absence of acetic acid, deglycosylation occurred as the major side reaction leading to decreased yield.[27] Even in the presence of acetic acid, deglycosylation accounted for variability in yield.

Using the same methods as presented in Scheme 1, a corresponding series of 6-anilin-*N*-yl analogues, **15**, **16**, **18**, **19**, and **21**−**23** was also prepared.

The 5′-deoxyribose analogue **17** was prepared starting with a DBU/TMSOTf-mediated glycoslyation[28] between 6-chloropurine (**24**) and 5-deoxy-sugar derivative **25**[29] (Scheme 2).[24] This reaction gave nucleoside **26a** as a 1:2 α/β-anomeric mixture[30] as evidenced by ^{1}H NMR ($CDCl_3$) integration of the anomeric protons at δ 6.72 and 6.13, respectively. These anomers were difficult to separate chromatographically at this stage. Removal of the acetates and reprotection resulted in bis-TBS derivative **26c** ready for C8 lithiation. Deprotonation with LICA and quenching with iodine provided the iodide **27** which was purified to a single anomer. Reaction of **27** with the potassium salt of indolin-*N*-yl anion produced a C6 adduct. This was reacted with the lithium salt of anilin-*N*-yl anion to provide a 6,8-disubstituted nucleoside, which provided **28** after desilylation.

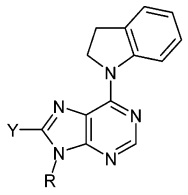
Conformational Assignment. When unsubstituted at C8, purine nucleosides favor an anti glycosyl bond orientation as the lowest energy conformation.[11,12] Substitution at C8 often induces a change in this conformational preference to syn because of nonbonded repulsions between the C8 substituent and the ribose ring.[13,14] Comparison of ^{1}H NMR spectroscopy of the 8-substituted purine nucleosides reported herein revealed differences in the syn−anti glycosyl bond conformational preferences that were dependent on the nature of the C8 substituent. The chemical shift values of the common protons of a select series of analogues are presented in Table 3 (see Supporting Information for a table of common proton chemical shift values for all analogues). Among these common protons, the H2′ resonance is consistently influenced by the glycosyl bond

Scheme 2[a]



[a] (a) TMSOTf, DBU, ACN; (b) NH_3, MeOH; (c) TBS−Cl, imidazole, DMF; (d) 1. LICA, THF; 2. I_2; (e) 1. conditions (a) of Scheme 1 (61%); 2. conditions (d) of Scheme 1 (82%); 3. conditions (c) of Scheme 1 (24%).

Table 3. ^{1}H NMR (δ) of Common Protons of Selected 6,8-Disubstituted Purine Nucleosides in DMSO-d_6[a]



R-groups
A, R = β-D-ribofuranosyl
B, R = 5'-deoxy-β-D-ribofuranosyl

	compound				
	5	**10b**	**9b**	**28**	**11b**
R	A	A	A	B	A
Y	H	anilin-*N*-yl	indolin-*N*-yl	anilin-*N*-yl	vinyl
conf[b]	anti	anti	syn	syn	anti
H2	8.46	8.33	8.43	8.34	8.44
H1′	5.97	6.22	5.82	6.03	6.05
H2′	4.61	4.67	5.15	5.2	4.8
H3′	4.17	4.18	4.21	4.23	4.19
H4′	3.97	4.08	3.98	3.95	4.00
5′-Hs	3.57, 3.68	3.76	3.6, 3.75	1.32	3.65
2′-OH	5.50	5.42	5.45	5.36	5.41
3′-OH	5.21	5.28	5.23	5.12	5.25
5′-OH	5.26	6.11	5.51	- -	5.48
$J_{H1'H2'}$ (Hz)	6	8	6	4	7

[a] Proton chemical shift values. Only centers of multiplets are reported. [b] Favored glycosyl bond conformation.

conformation and has been used as an indicator of a preference for either syn or anti conformation.[13,14] The H2′ chemical shift of δ 4.61 for the 8-H analogue **5** is typical of nucleosides in the anti conformation. Purine nucleosides in the syn conformation display H2′ values shifted significantly downfield compared to anti. This has been ascribed to a ring current deshielding effect and/or the magnetic anisotropy effect of the N3 atom,[13] since that portion of the base is proximal to H2′ in this conformation. Analogues **9b** and **28** with H2′ shifts > 0.5 ppm downfield of the H2′ value assigned to the 8-H analogue **5** are assigned as syn and are exemplary of most analogues presented in this report.

However, a few 8-substituted analogues can be assigned as having an anti glycosyl bond orientation. The 8-anilinyl compound **10b** is assigned as anti because its H2′ resonance, δ 4.67 (Table 3), is very similar to the value assigned to the H2′ of **5**. Another 8-anilinyl analogue **15** has a very similar ^{1}H NMR profile (Supporting Information) to **10b** and is also assigned as anti. Figure 1 depicts the conformational assignment of compounds **10b** and **15**. The observation that primary and secondary 8-aminopurine nucleosides favor the anti

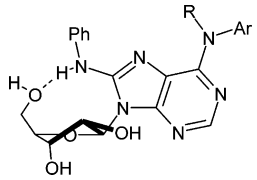


Figure 1. Representation of proposed hydrogen-bond stabilization of anti conformation of **10b** and **15** with 2′-endo (S-type) ribose ring pucker and gauche−gauche C4′−C5′ and gauche′−gauche′ C5′−O5′ bonds.

conformation was first noted by Miles et al. for 8-aminoguanosine derivatives.[31] Later, Evans and Kaplan through their studies on 8-amino-AMP analogues proposed that the anti conformation was stabilized by an unusual hydrogen bond between the C8 NH-group and the 5′-oxygen.[32] This hydrogen bond and its overall effect on glycosyl conformation of primary and secondary 8-aminopurine nucleosides and nucleotides, and influence on other factors of nucleoside and nucleotide conformation, have been extensively investigated by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopies, UV spectrosopy, and specific dehydrogenase enzyme binding studies.[33,34] X-ray crystallographic studies of 9-β-D-arabinofuranosyl-8-*n*-butylaminoadenine[35a] and 8-cyclopentylamino-N^6-ethyladenosine[35b,35c] provide convincing support for the secondary and primary 8-NH to 5′-O hydrogen bond. Consistent with these previous studies, the 8-anilin-*N*-yl purine nucleoside **10b** is assigned with a hydrogen bond between the C8 NH-group and the 5′-oxygen based on the significant (0.85 ppm) downfield shift of the 5′-OH compared to the C8-unsubstituted analogue **5** (δ 6.11 vs 5.26).[35a]

A hydrogen bond has not been observed in the reverse direction, i.e., 5′-OH to 8-N, since tertiary 8-aminopurine nucleosides and nucleotides were determined to have a syn glycosyl bond conformation.[31−33] Our studies corroborated this with the C8 tertiary amines **9b** and **16**, both of which had H2′ ^{1}H NMR resonances consistent with a syn conformation (see Table 3 and Supporting Information, respectively). Additionally, the 5′-deoxy analogue, **28**, favors a syn glycosyl bond conformation as well, as evidenced by its H2′ ^{1}H NMR chemical shift centered at δ 5.2. Lacking the hydrogen bond acceptor at the 5′-position, **28** cannot form an anti conformation-stabilizing hydrogen bond.

The H1′−H2′ coupling constant ($J_{H1'H2'}$) for the 8-anilin-*N*-yl nucleoside **10b** is unusually large for a nucleoside (8 Hz), but is consistent with previous primary and secondary 8-aminopurine nucleosides containing an anti conformation-stabilizing hydrogen bond[32−34,35b,35c] (Table

3). The magnitude of this coupling constant has been related to the percentage of 2′-endo ribose (S-type) ring pucker for a nucleoside, determined mathematically as $10J_{1'2'}$.[36] Thus the 8-anilin-*N*-yl nucleosides having an intramolecular hydrogen bond to stabilize the anti conformation, i.e., **10b** and **15**, have a high proportion (ca. 80%) of 2′-endo ribose ring pucker (Figure 1). This ribose ring pucker is presumably induced by the intramolecular 8-NH to 5′-O hydrogen bond and is supported by precedence of an X-ray crystal structure of a similar 8-aminoadenosine analogue.[35b,35c] However, it is notable that the published arabinose analogue solid-state structure displays a 3′-endo (N-type) ring pucker.[35a] The 5′-deoxy syn analogue **28** has a narrow $J_{1'2'}$ indicative of only ca. 40% 2′-endo ring pucker.

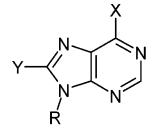
Additional NMR experiments would be necessary to confirm the exact nature of the C4′−C5′ and C5′−O5′ bond conformations for these syn and anti nucleosides. However, based on extensive studies previously reported for similar compounds,[14,33a,33c,34,35a−c] they will be assumed to be gauche−gauche and gauche′−gauche′, respectively.

8-Vinylpurine nucleosides have been reported previously but their glycosyl bond conformations were not assigned.[26b,26f] The H2′ NMR resonance of vinyl analogue **11b** is a multiplet centered at δ 4.8 (Table 3). The similarity of this resonance to the anti analogue **5** suggests that this 8-vinylpurine nucleoside has significant anti glycosyl bond character. The other 8-vinyl analogue, **22**, has a very similar ^{1}H NMR profile (see Supporting Information). The two 8-anilin-*N*-yl- and two 8-vinylpurine nucleosides (**10b**, **15**, **11b**, and **22**, respectively) are the only 8-substituted compounds of the present series with evidence of significant anti conformation, a factor which is relevant in discussions of AK inhibition SAR (vide supra).

Adenosine Kinase Inhibition. The purine nucleosides synthesized with various hydrophobic substituents at C6 and C8 were evaluated as inhibitors of AK (Table 4). The AK inhibition IC_{50} values were determined with human recombinant enzyme as previously described.[1a] Those analogues containing 6-indolin-*N*-yl substitution were, on average, 5-fold more potent AK inhibitors than the corresponding 6-anilin-*N*-yl analogues, based on seven pairs of compounds (see **10b**, **9b**, **17**, **8b**, **20**, **11b**, and **14b** compared to **15**, **16**, **18**, **19, 21**, **22**, and **23**, respectively). Furthermore, analogues having ^{1}H NMR evidence of an anti conformation, i.e. **10b** and **15** (both 8-anilin-*N*-yl) as well as **11b** (8-vinyl), are the most potent inhibitors of the series (AK IC_{50}s 0.019, 0.090, and 0.060 μM, respectively).

Analogues with syn glycosyl bonds are less inhibitory. An example is the 5′-deoxyribose analogue of **10b**, **28**, which is a 10-fold weaker inhibitor. Another syn compound, **9b**, with slightly larger C8 substitution (indolin-*N*-yl) than **10b** is a 100-fold weaker inhibitor. Substitution of an aryl group, furan-2-yl, onto C8 resulted in even less inhibitory compounds (e.g. **14b**, AK $IC_{50} = 2.9$ μM). Purine analogues with small C8 substitutents such as Cl, I, and acetylenyl (**17**, **8b**, and **13b**, respectively) also have syn conformations based on H2′ chemical shift values and are weak AKIs with IC_{50} values of 0.5−1 μM. Other analogues with small C8 substituents such as methylthio (**20**) and ethyl (**12b**) are moderately potent AKIs with IC_{50} values of 0.15 and 0.23 μM, respectively.

Table 4. Adenosine Kinase Inhibition



R-groups
A, R = β-D-ribofuranosyl
B, R = 5′-deoxy-β-D-ribofuranosyl

compd	R	X	Y	conf[a]	AK IC_{50} (μM)
10b	A	indolin-*N*-yl	anilin-*N*-yl	anti	0.019
15	A	anilin-*N*-yl	anilin-*N*-yl	anti	0.090
28	B	indolin-*N*-yl	anilin-*N*-yl	syn	0.20
9b	A	indolin-*N*-yl	indolin-*N*-yl	syn	2.0
16	A	anilin-*N*-yl	indolin-*N*-yl	syn	7.1
17	A	indolin-*N*-yl	Cl	syn	0.48
18	A	anilin-*N*-yl	Cl	syn	3.6
8b	A	indolin-*N*-yl	I	syn	1.0
19	A	anilin-*N*-yl	I	syn	4.5
20	A	indolin-*N*-yl	SMe	syn	0.15
21	A	anilin-*N*-yl	SMe	syn	1.0
12b	A	indolin-*N*-yl	ethyl	syn	0.23
11b	A	indolin-*N*-yl	vinyl	anti	0.060
22	A	anilin-*N*-yl	vinyl	anti	0.30
13b	A	indolin-*N*-yl	acetylenyl	syn	1.0
14b	A	indolin-*N*-yl	furan-2-yl	syn	2.9
23	A	anilin-*N*-yl	furan-2-yl	syn	6.9

[a] Favored glycosyl bond conformation.

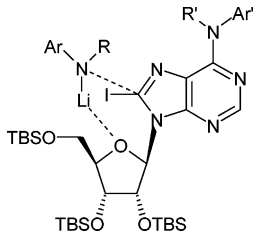


Figure 2. Proposed transition state depicting C8 substitution reaction with accelerating effect of ribose 4′-ether oxygen chelating to the lithium cation of the arylamino anion.

Discussion

While surveying different arylamino anion salts for the addition reactions on 6-chloro-8-iodopurine nucleoside **7a**, it was found that C6 vs C8 regioselectivity is possible and depends on the choice of alkali counterion. Potassium and sodium salts selectively add at the C6-chloro position while lithium salts displace halides at both C6 and C8. Stepwise reactions of potassium and then lithium salts of arylamino anions produce mixed 6,8-disubstituted purine nucleosides such as **10a** (Scheme 1).

Usually nucleophilic anions with potassium counterions are strong nucleophiles but intermolecular coordination with oxygen or nitrogen accelerates organolithium reactions.[37] Thus a possible explanation for the observed selectivity is that the proximal ribofuranose 4′-ether oxygen accelerates the C8 iodide substitution by chelating the lithium counterion of the arylamino anion (see Figure 2). Furthermore, the addition reaction is facilitated by rapid syn−anti equilibration[16] since the 8-iodo nucleoside substrates for this reaction, **8a**, **27**, and the precursor to **19**, are all expected to favor a syn conformation. The anti conformer reacts since the chelation results in the transfer of more electron density

to the nitrogen anion while it is in close proximity to C8. Since both 5′-hydroxy and 5′-deoxy analogues can be synthesized by this methodology, the 5′-O is not necessary and the ribofuranose 4′-ether oxygen is implicated as the primary source of rate acceleration.

A similar intramolecular coordination as depicted in Figure 1 is invoked to rationalize the superior AK inhibitory potency of 8-anilin-*N*-yl-substituted compounds **10b** and **15** (IC_{50}s = 0.019 and 0.090 μM, respectively). Solid-state structure determinations have demonstrated that AK binds inhibitors in an anti conformation.[15b] Consistent with this observation, in the current 6,8-disubstituted purine nucleoside series, compounds that favor an anti glycosyl bond conformation are the most potent AK inhibitors.[38] In fact, **10b** is a 9-fold more potent inhibitor compared to the best known purine-based AKI, 5′-deoxy-5′-aminoadenosine (**4**).[8] The compounds **10b** and **15** show ^{1}H NMR evidence for the formation of a hydrogen bond between the C8-NH and the 5′-O which stabilizes their anti glycosyl bond conformation (Table 3 and Supporting Information). Being constrained to the anti conformation, the 6- and 8-arylamino substituents can contribute optimally to binding of AK in a manner similar to the 4,5-diaryl groups of the diaryltubercidin series (for example, **1**). The importance of the solution anti glycosyl conformation is emphasized by comparing similar compounds such as **10b** and the C8-indolin-*N*-yl-substituted **9b**. Compound **9b**, without the C8-NH to 5′-O intramolecular hydrogen bond, has a syn conformation and is 100-fold less inhibitory. Furthermore, in the diaryltubercidin series, 5′-deoxy analogues have nearly identical inhibitory potency compared to their 5′-hydroxy counterparts.[1b] However in the current purine series, since it is unable to form a hydrogen bond to stabilize the anti conformation, the 5′-deoxy analogue **28** is syn and is a 10-fold weaker enzyme inhibitor compared to the corresponding 5′-hydroxy analogue **10b**.

Contrary to the SAR observed in the diaryltubercidin series, 6-indolin-*N*-yl substitution resulted in a ca. 5-fold more potent AKI compared to the corresponding 6-anilin-*N*-yl analogues (see Table 4). In the diaryltubercidin series, substitution of indoline for aniline at C4 resulted in 500-fold weaker inhibition (compare compounds **1** and **3** in Table 1). Thus with purine nucleosides, the C6 indoline group has a conformation relative to the purine ring allowing it to have enhanced hydrophobic interactions with the active site.

A few of these 6,8-disubstituted purine nucleosides which cannot form a hydrogen bond to stabilize an anti conformation were moderate inhibitors of AK with IC_{50} values as low as 0.15 μM. Examples include **28**, **12b**, and **20**, each with ^{1}H NMR indicative of a syn conformation (see Table 3 and Supporting Information). Moreover, syn−anti equilibration[16] is expected to make a significant population of anti conformer available for inhibition of AK. Yet these analogues are only moderate inhibitors because of the overall low proportion of their anti conformer in solution.

Interestingly, each of the C8 vinyl-substituted analogues **11b** and **22** display ^{1}H NMR evidence of an anti conformation without a stabilizing H-bond. This is unusual because while 8-unsubstituted purine nucleosides prefer the anti conformation,[11] 8-substituted nucleosides have been determined to favor the syn conformation[11−14] in order to relieve steric congestion between the C8 substitutent and the ribose ring. Presumably, the vinyl group is not as sterically demanding as the other substituents studied and does not induce a syn conformation. This combined with a favorable binding interaction of the vinyl group contribute to analogue **11b** being a good inhibitor of AK (IC_{50} = 0.060 μM).

Conclusions

A new two-step process has been discovered to selectively substitute 6,8-dihalopurine nucleosides with arylamino groups via the potassium and lithium salts of the amino anions. One product of this process, 6,8-bisarylaminopurine nucleoside **10b**, is the most potent purine-based AKI (IC_{50} = 0.019 μM) reported thus far and is proposed to bind to AK in a manner similar to the diaryltubercidin inhibitor series. AKI potency appears to be related to glycosyl bond conformational preference with those compounds favoring an anti conformation being the best inhibitors of AK. Finally, this study demonstrated that the ribose oxygens can have important effects on reaction selectivity and final solution conformation by way of both inter- and intramolecular coordination effects. Consideration of these effects can lead to new reactions and enzyme inhibitors, respectively.

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. *N*-Isopropylcyclohexylamine, indoline, and aniline were distilled under reduced pressure from CaH_2 and stored over KOH. 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 spectra were obtained on a Varian Gemini-200 operating at 200 MHz and spectra were recorded in units δ with tetramethylsilane (δ 0.00) as a reference line internal standard. All OH and NH proton chemical shifts were confirmed by D_2O exchange. 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.

4-Indolin-1-yl-5-phenyl-7-(5-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (3). This compound was prepared using methods previously described.[1b] Compound **3**: mp 173−175 °C; ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, J = 6 Hz, 5′-methyl), 2.81 (t, 2H, J = 9 Hz, indolinyl C3 Hs), 3.71 (t, 2H, J = 9 Hz, indolinyl Hs), 3.90 (m, 2H, H3′ and H4′), 4.51 (m, 1H, H2′), 5.11 (m, 1H, 3′-OH), 5.39 (d, 1H, J = 4 Hz, 2′-OH), 6.19 (d, 1H, J = 4 Hz, H1′), 6.62 (t, 1H, J = 8 Hz, phenyl H4), 6.79 (t, 1H, J = 8 Hz, indolinyl H5), 7.0−7.2 (m, 5H, indolinyl H4, H6, H7 and phenyl H3s), 7.48 (d, 1H, J = 8 Hz, phenyl H2s), 7.79 (s, 1H, H6), 8.52 (s, 1H, H2). Anal. ($C_{25}H_{24}N_4O_3$) C, H, N.

6-Indolin-*N*-yl-9-(β-D-ribofuranosyl)purine (5). A suspension of 6-chloropurine riboside (1.15 g, 4 mmol), indoline (0.58 mL, 5.2 mmol) and triethylamine (1 mL, 7.2 mmol) in 16 mL of *n*-BuOH was refluxed for 24 h. The solvent was evaporated and the residue triturated with 30 mL of refluxing ethanol. After cooling to room temperature, the solid was collected by filtration to provide 1.27 g (86%) of **5** as a white solid: mp 195−198 °C; ^{1}H NMR (DMSO-d_6) δ 3.27 (t, 2H, J = 9 Hz, indolinyl C3 Hs), 3.57 (ddd, 1H, J = 12, 6, 2 Hz, H5′),

3.68 (dt, 1H, J = 12, 2 Hz, H5′) 3.97 (q, 1H, J = 2 Hz, H4′), 4.17 (q, 1H, J = 5 Hz, H3′), 4.61 (q, 1H, J = 6 Hz, H2′), 4.77 (t, 2H, J = 9 Hz, indolinyl Hs), 5.21 (d, 1H, J = 5 Hz, 3′-OH), 5.26 (t, 1H, J = 5 Hz, 5′-OH), 5.50 (d, 1H, J = 6 Hz, 2′-OH), 5.97 (d, 1H, J = 6 Hz, H1′), 6.99 (t, 1H, J = 6 Hz, indolinyl H5), 7.19 (t, 1H, J = 9 Hz, indolinyl H6), 7.29 Hz, (d, 1H, J = 6 Hz, indolinyl H4), 8.46 (s, 1H, H2), 8.52 (s, 1H, H8), 8.61 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{18}H_{19}N_5O_4 \cdot 0.5H_2O$) C, H, N.

6,8-Dichloro-9-(2,3,5-tris-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (7b). To a solution of *N*-isopropylcyclohexylamine (0.20 mL, 1.2 mmol) in 8 mL of THF at −78 °C was added 0.84 mL of a 1.4 M solution of *n*-BuLi in hexanes (1.2 mmol), and the resulting mixture was stirred for 15 min. Then a solution of nucleoside **6** (500 mg, 0.79 mmol) in 5 mL of THF was added via cannula needle and the resulting pale orange solution stirred at −78 °C for 15 min. Then a solution of hexachloroethane (284 mg, 1.2 mmol) in 5 mL of THF was added via cannula needle. After stirring for 1 h at −78 °C, the solution was diluted with aqueous NH_4Cl and ether. The layers were separated, and the organic layer was washed with 5% HCl, water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with hexane/EtOAc mixtures of 100/1 and 75/1 to provide 450 mg (86%) of compound **7b** as a viscous oil: ^{1}H NMR (DMSO-d_6) δ −0.22−0.16 (m, 18H, TBS Me groups), 0.64 (s, 9H, *t*-Bu), 0.80 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 3.67 (dd, 1H, J = 11, 4 Hz, H5′), 3.90 (dd, 1H, J = 11, 4 Hz, H5′), 4.00 (q, 1H, J = 4 Hz, H4′), 4.81 (t, 1H, J = 4 Hz, H3′), 5.33 (t, 1H, J = 4 Hz, H2′), 5.87 (d, 1H, J = 4 Hz, H1′), 8.85 (s, 1H, H2). Anal. ($C_{28}H_{52}Cl_2N_4O_4Si_3$) C, H, N.

6-Chloro-8-methylthio-9-(2,3,5-tris-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (7c). Using the procedure descibed for the preparation of **7b**, with substitution of methyl methanesulfonate for hexachloroethane, provided compound **7c** (79% yield) as a viscous oil: ^{1}H NMR (DMSO-d_6) δ −0.33−0.14 (m, 18H, TBS Me groups), 0.73 (s, 9H, *t*-Bu), 0.74 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 2.78 (s, 3H, −SMe), 3.70 (dd, 1H, J = 11, 4 Hz, H5′), 3.8−4.0 (m, 1H, H5′), 3.9−4.0 (m, 1H, H4′), 4.62 (t, 1H, J = 4 Hz, H3′), 5.28 (t, 1H, J = 5 Hz, H2′), 5.80 (d, 1H, J = 5 Hz, H1′), 8.67 (s, 1H, H2).

6-Indolin-*N*-yl-8-iodo-9-(2,3,5-tris-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (8a). To a solution of indoline (1.25 mL, 11.1 mmol) in 50 mL of THF at −20 °C was added 11.1 mL of a 1.0 M solution of potassium *tert*-butoxide in THF (11.1 mmol). After stirring the mixture at −20 °C for 5 min, purine **7a** (4.20 g, 5.56 mmol) in 20 mL of THF was added via cannula needle. The resulting mixture was stirred at −20 °C for 1 h and then diluted with aqueous NH_4Cl and ether. The organic layer was separated, washed with 5% HCl, water, and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with hexane/EtOAc mixtures of 100/1 and 75/1 to provide 3.30 g (73%) of compound **8a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.03−0.16 (m, 18H, TBS Me groups), 0.76 (s, 9H, *t*-Bu), 0.81 (s, 9H, *t*-Bu), 0.95 (s, 9H, *t*-Bu), 3.25 (m, 2H, indolinyl C3 Hs), 3.71 (dd, 1H, J = 10, 4 Hz, H5′), 3.98 (m, 1H, H4′), 4.10 (dd, 1H, J = 10, 7 Hz, H5′), 4.57 (dd, 1H, J = 6, 4 Hz, H3′), 4.70 (m, 2H, indolinyl C2 Hs), 5.65 (dd, 1H, J = 6, 4 Hz, H2′), 5.88 (d, 1H, J = 6 Hz, H1′), 7.02 (t, 1H, J = 8 Hz, indolinyl H6), 7.23 (t, 1H, J = 8 Hz, indolinyl H7), 7.31 (d, 1H, J = 8 Hz, indolinyl H4), 8.38 (s, 1H, H2), 8.60 (d, 1H, J = 8 Hz, indolinyl H7).

6-Indolin-*N*-yl-8-iodo-9-(β-D-ribofuranosyl)purine (8b). Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b** (vide infra), compound **8a** was converted to compound **8b** (28%) and obtained as a white solid: mp 226−227 °C; ^{1}H NMR (DMSO-d_6) δ 3.28 (t, 2H, J = 9 Hz, indolinyl C3 Hs), 3.54 (m, 1H, H5′), 3.71 (dt, 1H, J = 12, 4 Hz, H5′), 3.99 (m, 1H, H4′), 4.24 (dd, 1H, J = 5, 7 Hz, H3′), 4.73 (m, 2H, indolinyl C2 Hs), 5.19 (q, 1H, J = 6 Hz, H2′), 5.25 (d, 1H, J = 4 Hz, 3′-OH), 5.36 (q, 1H, J = 4 Hz, 5′-OH), 5.49 (d, 1H, J = 6 Hz, 2′-OH), 5.83 (d, 1H, J = 6 Hz, H1′), 7.02 (t, 1H, J = 8 Hz, indolinyl H6), 7.23 (t, 1H, J = 8 Hz, indolinyl H7), 7.31 (d, 1H, J = 8 Hz, indolinyl H4), 8.39 (s, 1H, H2), 8.59 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{18}H_{18}IN_5O_4 \cdot 0.25H_2O$) C, H, N.

Compound **19** was prepared in a similar manner.

6,8-Bis(indolin-*N*-yl)-9-(2,3,5-tris-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (9a). To a solution of indoline (0.060 mL, 0.52 mmol) in 2 mL of THF at −20 °C was added 0.36 mL of a 1.4 M solution of *n*-BuLi in hexanes (0.52 mmol), and the mixture was stirred at −20 °C for 5 min. Then purine **7a** (200 mg, 0.26 mmol) in 1 mL of THF was added via cannula needle. The resulting mixture was stirred at −20 °C for 1 h and then diluted with aqueous NH_4Cl and ether. The organic layer was separated and washed with 5% HCl, water, and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with hexane/EtOAc mixtures of 100/1 and 75/1 to provide 100 mg (46%) of compound **9a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.08−0.12 (m, 18H, TBS Me groups), 0.73 (s, 9H, *t*-Bu), 0.86 (s, 9H, *t*-Bu), 0.97 (s, 9H, *t*-Bu), 3.1−3.3 (m, 4H, indolinyl C3 Hs), 3.74 (q, 1H, J = 5 Hz, H5′), 3.9−4.2 (m, 4H, H5′, H4′ and indolinyl C2 Hs), 4.53 (m, 1H, H3′), 4.66 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.68 (dd, 1H, J = 6, 5 Hz, H2′), 5.88 (d, 1H, J = 6 Hz, H1′), 6.8−7.4 (m, 6H, indolinyl Hs), 8.43 (s, 1H, H2), 8.57 (d, 1H, J = 8 Hz, indolinyl H7). MS calcd for $C_{44}H_{68}N_6O_4Si_3$: M + 1 = 829; M + 1 found 829.

6,8-Bis(indolin-*N*-yl)-9-(β-D-ribofuranosyl)purine (9b). Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b** (vide infra), compound **9a** was converted to compound **9b** (67%) as a white solid: mp 183 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.23 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.5−3.7 (m, 1H, H5′), 3.75 (dt, 1H, J = 12, 4 Hz, H5′), 3.98 (m, 1H, H4′), 4.21 (m, 1H, H3′), 4.08 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 4.68 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.15 (q, 1H, J = 6 Hz, H2′), 5.23 (d, 1H, J = 4 Hz, 3′-OH), 5.45 (d, 1H, J = 6 Hz, 2′-OH), 5.51 (dd, 1H, J = 4 Hz, 5′-OH), 5.82 (d, 1H, J = 6 Hz, H1′), 6.9−7.3 (m, 7H, indolinyl Hs), 8.43 (s, 1H, H2), 8.58 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{26}H_{26}N_6O_4 \cdot 0.25H_2O$) C, H, N.

8-Anilin-*N*-yl-6-indolin-*N*-yl-9-(2,3,5-tris-*O-tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (10a). To a solution of aniline (0.065 mL, 0.72 mmol) in 2 mL of THF at −20 °C was added 0.50 mL of a 1.4 M solution of *n*-BuLi in hexanes (0.72 mmol) and the mixture stirred at −20 °C for 15 min. Then purine **8a** (200 mg, 0.25 mmol) in 1 mL of THF was added via cannula needle. The resulting mixture was warmed and allowed to stir at room temperature for 16 h, then diluted with aqueous NH_4Cl and ether. The organic layer was separated and washed with 5% HCl, water, and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with hexane/EtOAc mixtures of 75/1, 50/1, and 35/1 to provide 134 mg (62%) of compound **10a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.09−0.16 (m, 18H, TBS Me groups), 0.73 (s, 9H, *t*-Bu), 0.81 (s, 9H, *t*-Bu), 0.95 (s, 9H, *t*-Bu), 3.25 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.7−3.9 (m, 1H, H5′), 4.0−4.2 (m, 2H, H5′, H4′), 4.5−4.6 (m, 1H, H3′), 4.68 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.45 (t, 1H, J = 6 Hz, H2′), 6.08 (d, 1H, J = 6 Hz, H1′), 6.94 (t, 1H, J = 8 Hz, anilinyl H4), 7.00 (t, 1H, J = 8 Hz, indolinyl H5), 7.19 (t, 1H, J = 8 Hz, indolinyl H6), 7.26 Hz, (d, 1H, indolinyl H4), 7.33 (t, 2H, J = 8 Hz, anilinyl H3), 7.72 (d, 2H, J = 8 Hz, anilinyl H2), 8.30 (s, 1H, H2), 8.49 (d, 1H, J = 8 Hz, indolinyl H7).

General Procedure for Fluoride Ion-Mediated Desilylation. 8-Anilin-*N*-yl-6-indolin-*N*-yl-9-(β-D-ribofuranosyl)-purine (10b). To a solution of purine **10a** (134 mg, 0.17 mmol) in 1.7 mL of DMF were added acetic acid (0.039 mL, 0.68 mmol) and tetraethylammonium fluoride (167 mg, 1.0 mmol). After stirring at room temperature for 16 h, the mixture was diluted with 30 mL of water, 30 mL of CH_2Cl_2, and 10 mL of methanol. The organic layer was separated and washed with brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with CH_2Cl_2/MeOH mixtures of 50/1 and 25/1 to provide a residue that was dissolved in 0.5 mL of MeOH and diluted with 5 mL of water

which resulted in precipitation of the product. Filtration and drying at 85 °C/0.1 mmHg for 1 h provided 46 mg (59%) of compound **10b** as a white solid: mp 260 °C; ^{1}H NMR (DMSO-d_6) δ 3.27 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.76 (m, 2H, C5′ Hs), 4.08 (m, 1H, H4′), 4.18 (m, 1H, H3′), 4.67 (q, 1H, J = 6 Hz, H2′), 4.76 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.28 (d, 1H, J = 4 Hz, 3′-OH), 5.42 (d, 1H, J = 7 Hz, 2′-OH), 6.11 (br s, 1H, 5′-OH), 6.22 (d, 1H, J = 8 Hz, H1′), 6.95 (t, 1H, J = 8 Hz, anilinyl H4), 7.00 (t, 1H, J = 8 Hz, indolinyl H5), 7.20 (t, 1H, J = 8 Hz, indolinyl H6), 7.27 Hz, (d, 1H, indolinyl H4), 7.34 (t, 2H, J = 8 Hz, anilinyl H3), 7.85 (d, 2H, J = 8 Hz, anilinyl H2), 8.33 (s, 1H, H2), 8.51 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{24}H_{24}N_6O_4$) C, H, N.

Compound **16** was prepared in a similar manner.

6-Indolin-*N*-yl-8-vinyl-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (11a). Nitrogen was bubbled for 5 min through a mixture containing iodopurine **8a** (350 mg, 0.42 mmol), vinyltributyltin (0.61 mL, 2.1 mmol), and tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.021 mmol) in 8 mL of DMF at room temperature. Then the mixture was heated for 2 h at 80 °C. After cooling to room temperature, the mixture was diluted with ethyl ether, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2, eluting with hexane/EtOAc mixtures of 75/1 and 50/1 to provide 260 mg (84%) of compound **11a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.44−0.14 (m, 18H, TBS Me groups), 0.67 (s, 9H, *t*-Bu), 0.86 (s, 9H, *t*-Bu), 0.94 (s, 9H, *t*-Bu), 3.25 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.7−3.8 (m, 1H, H5′), 3.9−4.1 (m, 2H, H4′, H5′), 4.41 (m, 1H, H3′), 4.82 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 4.96 (dd, 1H, J = 7, 4 Hz, H2′), 5.68 (d, 1H, J = 12 Hz, vinyl H2), 6.14 (d, 1H, J = 7 Hz, H1′), 6.47 (d, 1H, J = 12 Hz, vinyl H2), 7.01 (t, 1H, J = 8 Hz, indolinyl H5), 7.1−7.3 (m, 3H, indolinyl H4 and H6; vinyl H1), 8.45 (s, 1H, H2), 8.64 (d, 1H, J = 8 Hz, indolinyl H7).

6-Indolin-*N*-yl-8-vinyl-9-(β-D-ribofuranosyl)purine (11b). Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, compound **11a** was converted to compound **11b** (46%) as a tan solid: mp 193/198 °C; ^{1}H NMR (DMSO-d_6) δ 3.30 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.5−3.8 (m, 2H, C5′ Hs), 4.00 (m, 1H, H4′), 4.19 (br. s, 1H, H3′), 4.85 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 4.7−4.9 (m, 1H, H2′), 5.25 (d, 1H, J = 4 Hz, 3′-OH), 5.41 (d, 1H, J = 7 Hz, 2′-OH), 5.48 (dd, 1H, J = 7, 5 Hz, 5′-OH), 5.73 (d, 1H, J = 12 Hz, vinyl H), 6.05 (d, 1H, J = 7 Hz, H1′), 6.44 (d, 1H, J = 15 Hz, vinyl H), 7.0−7.4 (m, 3H, vinyl H and indolinyl H4, H6), 7.02 (t, 1H, J = 8 Hz, indolinyl H5), 8.44 (s, 1H, H2), 8.64 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{20}H_{21}N_5O_4\cdot0.25H_2O$) C, H, N.

Compound **22** was prepared in a similar manner.

8-Ethyl-6-indolin-*N*-yl-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (12a). A mixture of purine **11a** (145 mg, 0.20 mmol) and 10% Pd/C in 5 mL of MeOH and 5 mL of EtOAc was shaken under 45 psi H_2 in a Parr apparatus. After 2 h the mixture was filtered and evaporated. The residue was subjected to chromatography on SiO_2, eluting with 50:1 hexane/EtOAc to provide 118 mg (80%) of compound **12a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.42−0.13 (m, 18H, TBS Me groups), 0.70 (s, 9H, *t*-Bu), 0.80 (s, 9H, *t*-Bu), 0.92 (s, 9H, *t*-Bu), 1.32 (t, 3H, J = 8 Hz, Et), 2.91 (q, 2H, J = 8 Hz, Et), 3.24 (m, 2H, indolinyl C3 Hs), 3.7−3.8 (m, 1H, H5′), 3.9−4.1 (m, 2H, H4′, H5′), 4.49 (m, 1H, H3′), 4.76 (m, 2H, indolinyl C2 Hs), 5.43 (t, 1H, J = 4 Hz, H2′), 5.86 (d, 1H, J = 6 Hz, H1′), 6.97 (t, 1H, J = 8 Hz, indolinyl H5), 7.20 (t, 1H, J = 8 Hz, indolinyl H6). 7.27 (d, 1H, J = 8 Hz, indolinyl H4), 8.38 (s, 1H, H2), 8.58 (d, 1H, J = 8 Hz, indolinyl H7).

8-Ethyl-6-indolin-*N*-yl-9-(β-D-ribofuranosyl)purine (12b). Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, compound **12a** was converted to compound **12b** (35%) as a tan solid: mp 183−185 °C; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H, J = 7 Hz, Et), 2.96 (q, 2H, J = 7 Hz, Et), 3.27 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.5−3.7 (m, 1H, H5′), 3.72 (dt, 1H, J = 12, 4 Hz, H5′), 4.01 (m, 1H, H4′), 4.19 (br. s, 1H, H3′), 4.80 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 4.97 (q, 1H, J = 7 Hz, H2′), 5.28 (d, 1H, J = 5 Hz, 3′-OH), 5.42 (d, 1H, J = 7 Hz, 2′-OH), 5.66 (dd, 1H, J = 8, 4 Hz, 5′-OH), 5.85 (d, 1H, J = 7 Hz, H1′), 7.00 (t, 1H, J = 8 Hz, indolinyl H6), 7.23 (t, 1H, J = 8 Hz, indolinyl H5), 8.40 (s, 1H, H2), 8.61 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{20}H_{23}N_5O_4$) C, H, N.

6-Indolin-*N*-yl-8-(trimethylsilylacetylenyl)-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (13a). Nitrogen was bubbled for 5 min through a mixture containing iodopurine **8a** (302 mg, 0.36 mmol), trimethylsilylacetylene (0.31 mL, 2.2 mmol), copper(I) iodide (4 mg, 0.02 mmol), and bis(triphenylphosphine)palladium(II) chloride (6 mg, 0.01 mmol) in 7 mL of triethylamine at room temperature. Then the mixture was heated for 2 h at 80 °C. After cooling to room temperature, the mixture was filtered and evaporated. The residue was triturated with ethyl ether, and again the resulting mixture was filtered and evaporated. The residue was subjected to chromatography on SiO_2, eluting with hexane/EtOAc mixtures of 100:1 and 75:1 to provide 179 mg (61%) of **13a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.12−0.29 (m, 27H, TBS and TMS Me groups), 0.74 (s, 9H, *t*-Bu), 0.88 (s, 9H, *t*-Bu), 0.94 (s, 9H, *t*-Bu), 3.28 (m, 2H, indolinyl C3 Hs), 3.77 (m, 1H, H5′), 4.0 (m, 1H, H5′), 4.1−4.2 (m, 1H, H4′), 4.45 (d, 1H, J = 4 Hz, H3′), 4.76 (m, 2H, indolinyl C2 Hs), 5.47 (dd, 1H, J = 7, 4 Hz, H2′), 6.09 (d, 1H, J = 7 Hz, H1′), 7.04 (t, 1H, J = 8 Hz, indolinyl H5), 7.25 (t, 1H, J = 8 Hz, indolinyl H6), 7.33 Hz, (d, 1H, indolinyl H4), 8.50 (s, 1H, H2), 8.64 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{41}H_{69}N_5O_4Si_4$) C, H, N.

8-Acetylenyl-6-indolin-*N*-yl-9-(β-D-ribofuranosyl)purine (13b). Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, compound **13a** was converted to compound **13b** (40%) as a tan solid: mp 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 3.29 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.4−3.8 (m, 2H, C5′ Hs), 4.00 (m, 1H, H4′), 4.2−4.3 (m, 1H, H3′), 4.76 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.10 (q, 1H, J = 6 Hz, H2′), 5.28 (d, 1H, J = 6 Hz, 3′-OH), 5.49 (t, 1H, J = 6 Hz, 5′-OH), 5.51 (d, 1H, J = 6 Hz, 2′-OH), 6.02 (d, 1H, J = 6 Hz, H1′), 7.05 (t, 1H, J = 8 Hz, indolinyl H6), 7.26 (t, 1H, J = 8 Hz, indolinyl H5), 7.33 (d, 1H, J = 8 Hz, indolinyl H4), 8.51 (s, 1H, H2), 8.64 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{20}H_{19}N_5O_4\cdot0.25H_2O$) C, H, N.

8-Furan-2-yl-6-indolin-*N*-yl-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (14a). Nitrogen was bubbled for 5 min through a mixture containing iodopurine **8a** (200 mg, 0.25 mmol), 2-(tributylstannyl)furan (0.31 mL, 0.99 mmol), copper(II) oxide (20 mg, 0.25 mmol), and tetrakis-(triphenylphosphine)palladium(0) (14 mg, 0.013 mmol) in 5 mL of DMF at room temperature. Then the mixture was heated for 2 h at 100 °C. After cooling to room temperature, the mixture was filtered, diluted with ethyl ether, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2, eluting with hexane/EtOAc mixtures of 50/1, 30/1 and 15/1 to provide 162 mg (83%) of compound **11a** as a foam: ^{1}H NMR (DMSO-d_6) δ −0.37−0.16 (m, 18H, TBS Me groups), 0.71 (s, 9H, *t*-Bu), 0.78 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 3.36 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.71 (dd, 1H, J = 11, 7 Hz, H5′), 3.9−4.1 (m, 2H, H5′, H4′), 4.69 (m, 1H, H3′), 4.85−4.95 (m, 2H, indolinyl C2 Hs), 5.64 (t, 1H, J = 5 Hz, H2′), 6.21 (d, 1H, J = 6 Hz, H1′), 6.60 (m, 1H, furanyl H4), 7.02 (t, 1H, J = 8 Hz, indolinyl H5), 7.14 (d, 1H, J = 4 Hz, furanyl H3), 7.24 (t, 1H, J = 8 Hz, indolinyl H6), 7.31 Hz, (d, 1H, indolinyl H4), 8.01 (s, 1H, furanyl H5), 8.46 (s, 1H, H2), 8.64 (d, 1H, J = 8 Hz, indolinyl H7).

8-Furan-2-yl-6-indolin-*N*-yl-9-(β-D-ribofuranosyl)purine (14b). Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, compound **14a** was converted to compound **14b** (16%) as a white solid: mp 170−173 °C; ^{1}H NMR (DMSO-d_6) δ 3.29 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.4−3.8 (m, 2H, C5′ Hs), 4.01 (m, 1H, H4′), 4.2−4.3 (m, 1H, H3′), 4.84 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.21 (q, 1H, J = 6 Hz, H2′), 5.24 (d, 1H, J = 4 Hz, 3′-OH), 5.48 (d, 1H, J = 6 Hz, 2′-OH), 5.4−5.6 (m, 1H, 5′-OH), 6.18 (d, 1H, J = 7 Hz, H1′), 6.81 (m, 1H, furanyl H4), 7.04 (t, 1H, J = 8 Hz, indolinyl H6), 7.23 (d, 1H, J = 4 Hz, furanyl H3), 7.31 (t, 1H, J = 8 Hz, indolinyl H5), 7.33 (d, 1H,

J = 8 Hz, indolinyl H4), 8.06 (s, 1H, furanyl H5), 8.48 (s, 1H, H2), 8.65 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{22}H_{21}N_5O_5 \cdot 0.5H_2O$) C, H, N.

Compound **23** was prepared in a similar manner.

6,8-Bis(anilin-*N*-yl)-9-(β-D-ribofuranosyl)purine (15). Step 1. To a solution of aniline (0.18 mL, 1.99 mmol) in 3.3 mL of THF at −20 °C was added 1.34 mL of a 1.48 M solution of *n*-BuLi in hexanes (1.99 mmol), and the mixture was stirred at −20 °C for 15 min. Then purine **7a** (250 mg, 0.33 mmol) in 1 mL of THF was added via cannula needle. The resulting mixture was stirred at −20 °C for 1 h (TLC showed C6 adduct and a trace of the 6,8-bis adduct). The mixture was stirred at 20 °C for 16 h and then diluted with aqueous NH_4Cl and ether. The organic layer was separated and washed with 5% HCl, water, and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2 and eluted with hexane/EtOAc mixtures of 50:1, 25:1, and 15:1 to provide 190 mg (74%) of 6,8-bis(anilin-*N*-yl)-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine as a foam: ^{1}H NMR (DMSO-d_6) δ −0.34−0.14 (m, 18H, TBS Me groups), 0.71 (s, 9H, *t*-Bu), 0.78 (s, 9H, *t*-Bu), 0.93 (s, 9H, *t*-Bu), 3.78 (dd, 1H, J = 11, 4 Hz, H5′), 4.0−4.2 (m, 2H, H5′, H4′), 4.56 (t, 1H, J = 4 Hz, H3′), 5.45 (t, 1H, J = 5 Hz, H2′), 6.04 (d, 1H, J = 5 Hz, H1′), 6.98 (t, 2H, J = 8 Hz, anilinyl H4s), 7.29 (t, 4H, J = 8 Hz, anilinyl H3s), 7.78 (d, 2H, J = 8 Hz, anilinyl H2), 7.84 (d, 2H, J = 8 Hz, anilinyl H2), 8.20 (s, 1H, H2), 9.10 (s, 1, NH), 9.18 (s, 1, NH).

Step 2. Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, 6,8-bis(anilin-*N*-yl)-9-(2,3,5-tris-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-purine was converted to **15** (50%) as a white solid: mp 140−145 °C; ^{1}H NMR (DMSO-d_6) δ 3.76 (m, 2H, C5′ Hs), 4.08 (m, 1H, H4′), 4.18 (m, 1H, H3′), 4.69 (q, 1H, J = 6 Hz, H2′), 5.28 (d, 1H, J = 4 Hz, 3′-OH), 5.42 (d, 1H, J = 7 Hz, 2′-OH), 6.08 (br s, 1H, 5′-OH), 6.18 (d, 1H, J = 8 Hz, H1′), 7.01 (t, 1H, J = 8 Hz, anilinyl H4), 7.02 (t, 1H, J = 8 Hz, anilinyl H4), 7.33 (t, 4H, J = 8 Hz, anilinyl H3), 7.87 (d, 2H, J = 8 Hz, anilinyl H2), 7.94 (d, 2H, J = 8 Hz, anilinyl H2), 8.25 (s, 1H, H2), 9.12 (s, 1, NH), 9.25 (s, 1, NH). Anal. ($C_{22}H_{22}N_6O_4 \cdot 0.25H_2O$) C, H, N.

6-Indolin-*N*-yl-8-chloro-9-(β-D-ribofuranosyl)purine (17). Using the general method for C6 indoline addition as described for the preparation of **8a** followed by the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, compound **7b** was converted to compound **17** (33% for 2 steps) as a white solid: mp 215 °C; ^{1}H NMR (DMSO-d_6) δ 3.28 (t, 2H, J = 9 Hz, indolinyl C3 Hs), 3.5−3.6 (m, 1H, H5′), 3.71 (dt, 1H, J = 12, 4 Hz, H5′), 3.9−4.0 (m, 1H, H4′), 4.2−4.3 (m, 1H, H3′), 4.69 (t, 2H, J = 9 Hz, indolinyl C2 Hs), 5.12 (q, 1H, J = 6 Hz, H2′), 5.2−5.3 (m, 2H, 5′-OH and 3′-OH), 5.54 (d, 1H, J = 6 Hz, 2′-OH), 5.92 (d, 1H, J = 7 Hz, H1′), 7.03 (t, 1H, J = 8 Hz, indolinyl H5), 7.24 (t, 1H, J = 8 Hz, indolinyl H6), 7.32 Hz, (d, 1H, J = 8 Hz, indolinyl H4), 8.49 (s, 1H, H2), 8.60 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{18}H_{18}ClN_5O_4$) C, H, N.

Compound **18** was prepared in a similar manner.

6-Indolin-*N*-yl-8-methylthio-9-(β-D-ribofuranosyl)-purine (20). Using the general method for C6 indoline addition as described for the preparation of **8a** followed by the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, compound **7c** was converted into compound **20** (48% for two steps) as a white solid: mp 129−131 °C; ^{1}H NMR (DMSO-d_6) δ 2.76 (s, 3H, −SMe), 3.28 (t, 2H, J = 9 Hz, indolinyl C3 Hs), 3.5−3.6 (m, 1H, H5′), 3.71 (dt, 1H, J = 12, 4 Hz, H5′), 3.9−4.1 (m, 1H, H4′), 4.1−4.3 (m, 1H, H3′), 4.81 (t, 2H, J = 9 Hz, indolinyl C2 Hs), 5.04 (q, 1H, J = 6 Hz, H2′), 5.27 (d, 1H, J = 4 Hz, 3′-OH), 5.41 (dd, 1H, J = 8, 4 Hz, 5′-OH), 5.47 (d, 1H, J = 6 Hz, 2′-OH), 5.78 (d, 1H, J = 6 Hz, H1′), 7.01 (t, 1H, J = 8 Hz, indolinyl H5), 7.23 (t, 1H, J = 8 Hz, indolinyl H6), 7.30 Hz, (d, 1H, J = 8 Hz, indolinyl H4), 8.39 (s, 1H, H2), 8.59 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{19}H_{21}N_5O_4S \cdot 0.5H_2O \cdot 0.4CH_3OH$) C, H, N.

Compound **21** was prepared in a similar manner.

6-Chloro-9-(5-deoxy-2,3-bis-*O*−*t*-butyldimethylsilyl-β-(and α-)-D-ribofuranosyl)purine (26c). Step 1. To a mixture of sugar **25**[29] (7.98 g, 30.6 mmol) and 6-chloropurine (**24**) (5.2 g, 33.6 mmol) in 61 mL of acetonitrile was added DBU (13.7 mL, 91.7 mmol). The solution was cooled to ice bath temperature, trimethylsilyl trifluoromethanesulfonate (19.5 mL, 100.9 mmol) added, and the resulting mixture then heated to 60 °C for 16 h. After cooling, the mixture was mixed thoroughly with aqueous saturated $NaHCO_3$ and CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic extracts were dried (Na_2SO_4) and evaporated. The residue was subjected to chromatography on SiO_2, eluting with 95:5 CH_2Cl_2/MeOH to provide 6.48 g (55%) of 6-chloro-9-(5-deoxy-2,3-bis-*O*-acetyl-β-(and α-)-D-ribofuranosyl)purine (**26a**).[30b]

Step 2. Nucleoside **26a** (6.00 g, 16.9 mmol) was stirred at room temperature in 125 mL of 25% NH_3 in MeOH for 3 h. The solvent was evaporated and the residue subjected to chromatography on SiO_2, eluting with EtOAc to provide 5.67 g of slightly impure 6-chloro-9-(5-deoxy-β-(and α-)-D-ribofuranosyl)purine (**26b**).[30b]

Step 3. A mixture of nucleoside **26b** (5.67 g), imidazole (9.97 g, 146 mmol) and TBDMS-Cl (15.78 g, 104 mmol) in 84 mL of DMF was stirred at room temperature for 16 h when more TBDMS-Cl (3.15 g, 21 mmol) and imidazole (1.42 g, 21 mmol) were added. The mixture was heated to 50 °C for 16 h then cooled to room temperature and diluted with ether and washed with 10% HCl, water, and brine, dried ($MgSO_4$), and evaporated. The residue was subjected to chromatography on SiO_2, eluting with 15:1 hexane/EtOAc to provide 3.82 g (35% after 2 steps) of the title compound **26c** as a white solid. β-Isomer: ^{1}H NMR ($CDCl_3$) δ −0.22−0.16 (m, 12H, TBS Me groups), 0.86 (s, 9H, *t*-Bu), 0.91 (s, 9H, *t*-Bu), 1.46 (d, 3H, J = 7 Hz, 5′-methyl), 3.9−4.0 (m, 1H, H4′), 4.28 (t, 1H, J = 7 Hz, H3′), 4.84 (t, 1H, J = 4 Hz, H2′), 5.91 (d, 1H, J = 4 Hz, H1′), 8.20 (s, 1H, H8), 8.74 (s, 1H, H2). α-Isomer selected peaks: ^{1}H NMR ($CDCl_3$) δ 6.48 (d, 1H, J = 6 Hz, H1′), 8.22 (s, 1H, H8), 8.84 (s, 1H, H2).

6-Chloro-8-iodo-9-(5-deoxy-2,3-bis-*O*−*t*-butyldimethylsilyl-β-D-ribofuranosyl)purine (27). Using the procedure described for the preparation of **7b**, with substitution of iodine for hexachloroethane, provided compound **27** (74%) as a viscous oil: ^{1}H NMR ($CDCl_3$) δ −0.35−0.16 (m, 12H, TBS Me groups), 0.83 (s, 9H, *t*-Bu), 0.98 (s, 9H, *t*-Bu), 1.42 (d, 3H, J = 7 Hz, 5′-methyl), 4.1−4.3 (m, 1H, H4′), 4.33 (t, 1H, J = 4 Hz, H3′), 5.38 (t, 1H, J = 5 Hz, H2′), 5.94 (d, 1H, J = 5 Hz, H1′), 8.68 (s, 1H, H2). MS calcd for $C_{22}H_{38}ClIN_4O_3Si_2$: M + 1 = 625/627; M + 1 found 625/627.

8-Anilin-*N*-yl-6-indolin-*N*-yl-9-(5-deoxy-β-D-ribofuranosyl)purine (28). Step 1. Using the general method for C6 indoline addition as described for the preparation of **8a**, compound **27** was converted into 6-indolin-*N*-yl-8-iodo-9-(5-deoxy-2,3-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-purine (61%): ^{1}H NMR ($CDCl_3$) δ −0.26 (s, 3H, TBS Me), −0.03 (s, 3H, TBS Me), 0.17 (s, 6H, TBS Me groups), 0.85 (s, 9H, *t*-Bu), 0.99 (s, 9H, *t*-Bu), 1.44 (d, 3H, J = 7 Hz, 5′-methyl), 3.30 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 4.1−4.2 (m, 1H, H4′), 4.45 (t, 1H, J = 3 Hz, H3′), 4.81 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.45 (t, 1H, J = 5 Hz, H2′), 5.86 (d, 1H, J = 5 Hz, H1′), 7.05 (t, 1H, J = 8 Hz, indolinyl H6), 7.25−7.29 (m, 2H, indolinyl H7 and H4), 8.43 (s, 1H, H2), 8.66 (d, 1H, J = 8 Hz, indolinyl H7).

Step 2. Using the general method for C8 aniline addition as described for the preparation of **10a**, 6-indolin-*N*-yl-8-iodo-9-(5-deoxy-2,3-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-purine was converted into 8-anilin-*N*-yl-6-indolin-*N*-yl-9-(5-deoxy-2,3-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-purine (82%): ^{1}H NMR ($CDCl_3$) δ −0.26 (s, 3H, TBS Me), −0.01 (s, 3H, TBS Me), 0.11 (s, 6H, TBS Me groups), 0.76 (s, 9H, *t*-Bu), 0.94 (s, 9H, *t*-Bu), 1.52 (d, 3H, J = 7 Hz, 5′-methyl), 3.28 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.97 (t, 1H, J = 4 Hz, H3′), 4.2−4.4 (m, 1H, H4′), 4.80 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.00 (t, 1H, J = 5 Hz, H2′), 6.08 (d, 1H, J = 5 Hz, H1′), 6.97 (t, 1H, J = 8 Hz, anilinyl H4), 7.05 (t, 1H, J = 8 Hz,

indolinyl H6), 7.2−7.3 (m, 2H, indolinyl H7 and H4), 7.37 (t, 2H, J = 8 Hz, anilinyl H3), 7.61 (d, 2H, J = 8 Hz, anilinyl H2), 7.71 (s, 1H, NH), 8.41 (s, 1H, H2), 8.51 (d, 1H, J = 8 Hz, indolinyl H7).

Step 3. Using the general method for fluoride ion-mediated desilylation described for the preparation of **10b**, 8-anilin-*N*-yl-6-indolin-*N*-yl-9-(5-deoxy-2,3-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine was converted to the title compound (**28**) (24%) as a tan solid: mp 252−253 °C; ^{1}H NMR (DMSO-d_6) δ 1.32 (d, 3H, J = 6 Hz, 5′-methyl), 3.26 (t, 2H, J = 8 Hz, indolinyl C3 Hs), 3.95 (m, 1H, H4′), 4.23 (m, 1H, H3′), 4.73 (t, 2H, J = 8 Hz, indolinyl C2 Hs), 5.12 (d, 1H, J = 4 Hz, 3′-OH), 5.15−5.25 (m, 1H, H2′), 5.36 (d, 1H, J = 4 Hz, 2′-OH), 6.03 (d, 1H, J = 4 Hz, H1′), 6.95 (t, 1H, J = 8 Hz, anilinyl H4), 6.98 (t, 1H, J = 8 Hz, indolinyl H5), 7.2−7.4 (m, 3H, indolinyl H4, H6 and anilinyl H3), 7.83 (d, 2H, J = 8 Hz, anilinyl H2), 8.34 (s, 1H, H2), 8.50 (d, 1H, J = 8 Hz, indolinyl H7). Anal. ($C_{24}H_{24}N_6O_3$) C, H, N.

Human Recombinant Adenosine Kinase IC_{50} Determination. This was performed as previously described.[1a]

Acknowledgment. We thank Mr. Juergen M. Schanzer for performing the adenosine kinase inhibition assay and Dr. C. E. Browne and M. M. Ramirez-Weinhouse for preliminary studies on purine nucleoside AKIs.

Supporting Information Available: A table of common ^{1}H NMR chemical shifts and a table of melting points and microanalysis data are available for all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM048968J