Adenosine Kinase Inhibitors. 3. Synthesis, SAR, and Antiinflammatory Activity of a Series of L-Lyxofuranosyl Nucleosides

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Chronic inflammatory diseases, such as arthritis and rheumatoid arthritis, remain major health problems worldwide. We previously demonstrated that adenosine kinase inhibitors (AKIs) exhibit antiinflammatory effects by inhibiting TNF-α production, neutrophil accumulation, and edema formation. Although adenosine receptor agonists produce similar effects, AKIs showed the antiinflammatory activity without the cardiovascular side effects that prevented the development of adenosine receptor specific agonists. However, previously described potent AKIs, such as 5-iodotubercidin, are nucleosides which have the potential to undergo in vivo 5′-*O*-phosphorylation and therefore produce cytotoxicity. In an effort to eliminate toxicities produced by phosphorylated nucleosides, L-lyxofuranosyl analogues of tubercidin were tested as potential AKIs since the opposite stereochemical orientation of the CH_2OH was expected to eliminate intracellular phosphorylation. Described herein are the discovery of a new series of AKIs based on α-L-lyxofuranosyl nucleosides, their SAR, as well as the antiinflammatory activity of the lead compound GP790 (IC_{50} = 0.47 nM, 47% inhibition of paw swelling at 10 mg/kg in rat carrageenan paw edema model). In addition, a study showing that in the skin lesion model the antiinflammatory activity is reversed by an A2 selective adenosine receptor antagonist 3,7-dimethyl-1-propylxanthine (DMPX) is also described.

Introduction

The protective responses produced by adenosine receptor activation present considerable therapeutic potential for both adenosine and its receptor agonists.[1] While adenosine itself is an approved treatment for supraventricular tachycardia, its short plasma half-life (<5 s) makes it unsuitable for most other diseases. Adenosine receptor agonists, on the other hand, exhibit a narrow therapeutic window which is attributed to a number of factors, including expression of receptors in the heart and the brain, which result in severe cardiovascular and CNS side effects. To circumvent these problems, efforts were focused on exploring the utility of the agents called adenosine regulating agents (ARAs) which are postulated to elevate endogenous adenosine in a site and event specific manner (e.g., ischemia, tissue injury, seizures, etc.). Endogenous adenosine is regulated by a number of adenosine metabolizing enzymes, including adenosine kinase (AK) which converts adenosine to adenosine-5′-monophosphate (AMP). Inhibition of this cytosolic enzyme increases intracellular concentrations of adenosine which is subsequently transported via adenosine transporters to the extracellular space. This increase in the local concentration of adenosine would activate nearby cell surface adenosine receptors leading to the desired pharmacological effect.[2] Furthermore, the short half-life of adenosine is expected to minimize the side effects by localizing it near the site of production.

We demonstrated previously that adenosine kinase inhibitors (AKIs) provide therapeutic benefits in animal models of various diseases.[3−8] For example, GP515 (**1a**, Chart 1), a potent AKI (IC_{50} = 4 nM) exhibits broad spectrum antiinflammatory activity both in vitro and in vivo, including the inhibition of TNF-α production, neutrophil accumulation, and edema formation in a dose dependent fashion without the accompanying hemodynamic side effects.[6−8] The antiinflammatory effects of GP515 were diminished by an A2 adenosine receptor antagonist,[8] suggesting that the in vivo activity is adenosine receptor mediated. It was also demonstrated that AKIs, such as 5-iodotubercidin[9] **1b** (IC_{50} = 26 nM) and 4-*N*-(4-fluorophenyl)-5-phenyl-5′-deoxytubercidin[2,10] **1c** (IC_{50} = 1.5 nM) exhibit antiseizure effects in rats subjected to maximum electroshock (MES) induced seizures in a theophylline reversible fashion.[1,2,5] These results are consistent with the elevation of endogenous adenosine levels by AK inhibition and subsequent adenosine receptor activation. It is noteworthy, however, that unlike adenosine receptor agonists, AKIs utilized in these studies did not cause hemodynamic side effects at doses up to 10-fold the ED_{50}. Therefore, AKIs represent an alternative to adenosine and adenosine receptor agonists for eliciting the potential therapeutic benefits from adenosine receptor activation.

Nucleoside kinases, such as AK, cytidine kinase, thymidine kinase, catalyze phosphorylation of a variety of nucleosides, including nucleoside-based drugs, giving rise to nucleoside-5′-*O*-phosphates which are incorporated into RNA or DNA via the corresponding triphosphates. Thus, 5′-*O*-phosphates of anticancer and antiviral nucleosides are responsible not only for the pharmacological activity but also for their cytotoxic side effects. A majority of the previously published AKIs[1] are

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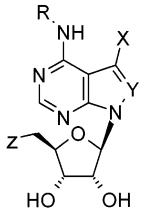
Chart 1



1a R = H; X = Br; Y = N; Z = NH_2
1b R = H; X = I; Y = CH; Z = OH
1c R = 4-F-phenyl; X = phenyl; Y = CH; Z = H

tubercidin analogues whose sugar moieties are identical to those in natural nucleosides and therefore have the potential to undergo 5′-*O*-phosphorylation. To reduce such risks in AK inhibitors, the current study is directed toward examining L-lyxofuranosyl nucleosides in which the stereochemical orientation of the 5′-CH_2OH group is opposite to that of the β-D-ribofuranosyl nucleosides. While this stereochemical arrangement of the group was designed to circumvent 5′-*O*-phosphorylation, it could potentially render the molecule inactive due to its misfit into the active site of AK. To our surprise, however, a number of L-lyxonucleosides exhibited AKI activity as potently as the corresponding ribofuranosyl analogues. Described here in are a new series of AKIs based on α-L-lyxofuranosyl nucleosides, their discovery and SAR, as well as the antiinflammatory activity of the lead compound GP790 (IC_{50} = 0.47 nM) in the carrageenan paw edema model of inflammation. In addition, a study showing that in the skin lesion model the antiinflammatory activity is reversed by an A2 selective adenosine receptor antagonist 3,7-dimethyl-1-propylxanthine (DMPX) is also described.

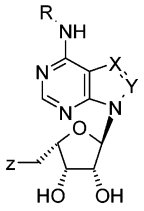
Chemistry

The compounds listed in Table 1 were synthesized via glycosylation of pyrrolo[2,3-*d*]pyrimidine, pyrazolo[3,4-*d*]pyrimidine, and adenine bases with appropriately protected L-lyxofuranoses using different glycosylation procedures. Thus, pyrrolo[2,3-*d*]pyrimidine bases (**10a**−**d**) were glycosylated with L-lyxofuranosyl chlorides **9a**−**c** using a previously developed tris-[2-(2-methoxyethoxy)ethyl]amine (TDA-1) mediated glycosylation method[2] (Schemes 2, 3), whereas, pyrazolo[3,4-*d*]pyrimidine and adenine bases were glycosylated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-L-lyxofuranose (**8**) using BF_3·Et_2O[11] and $SnCl_4$,[12] respectively (Scheme 4).

The carbohydrate precursors **4**−**6** and **8** used in various glycosylation experiments were synthesized as shown in Scheme 1. Due to the unavailability of L-lyxofuranose in the quantities needed for the present investigation, an efficient method was developed for its synthesis. Since D-ribose and L-lyxose differ only in the stereochemistry at the C4-position, it was envisioned that inversion of the CH_2OH group of D-ribose would provide L-lyxose. To accomplish this transformation, D-ribose was converted to **2** by the literature procedure[13] and subjected to hydroboration using BH_3·THF. The resulting product was found exclusively to be methyl 2,3-*O*-isopropylidene-L-lyxofuranoside **3**, which was identical by ^1H NMR spectroscopy to the one prepared from the commercial L-lyxose. Exclusive formation of **3** suggested that the attack of borane on **2** took place from the less hindered β-face of the ribofuranose ring. Complete deprotection of **3** with 0.1 N H_2SO_4 and reintroduction of the 2,3-*O*-isopropylidene and the 5-*O*-*tert*-butyldimethylsilyl groups using standard reaction conditions[2] furnished **4** in a 50% overall yield. The 5-*O*-methyl derivative **5** was prepared by alkylating **3** with MeI in the presence of NaH followed by the manipulation of the protecting groups as described for **4**.

5-Deoxy-2,3-*O*-isopropylidene-L-lyxofuranose **6** was derived from **2** by catalytic hydrogenation to give a 9:1 mixture of methyl 5-deoxy-2,3-*O*-isopropylidene-L-lyxofuranoside and the corresponding methyl 5-deoxy-2,3-*O*-isopropylidene-D-ribofuranoside. Complete deprotection of the product mixture followed by reprotection of

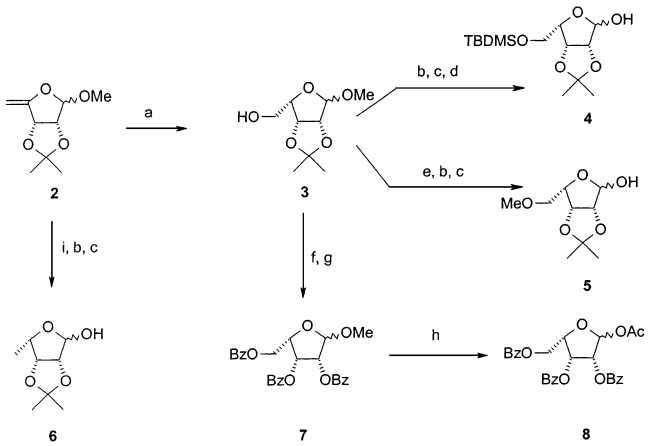
Table 1. AK Inhibition SAR



compd	R	X	Y	Z	IC_{50},[a] nM
1a[8]					4
1b[1]					26
1c[2]					5
13a	H	C−I	CH	OH	68
13b	H	C−Br	CH	OH	280
13c	H	C−I	CH	OCH_3	2000
13d	H	C−I	CH	H	400
13e	H	C−Br	CH	H	10000
16a	Ph	C−Ph	CH	OH	0.47
16b	*p*-F−Ph	C−Ph	CH	OH	1.5
16c	Ph	C−Ph	CH	OCH_3	15
16d	Ph	C−Ph	CH	H	0.8
19a	H	C−Br	N	OH	10000
19b	Ph	Ph	N	OH	2.8
21	H	N	CH	OH	1000
24a	H	C−I	CH	NH_2	16
24b	Ph	C−Ph	CH	NH_2	36
24c	H	N	CH	NH_2	10000
26	H	C−Br	N	NH_2	170
28	Ph	C−Ph	N	NH_2	42

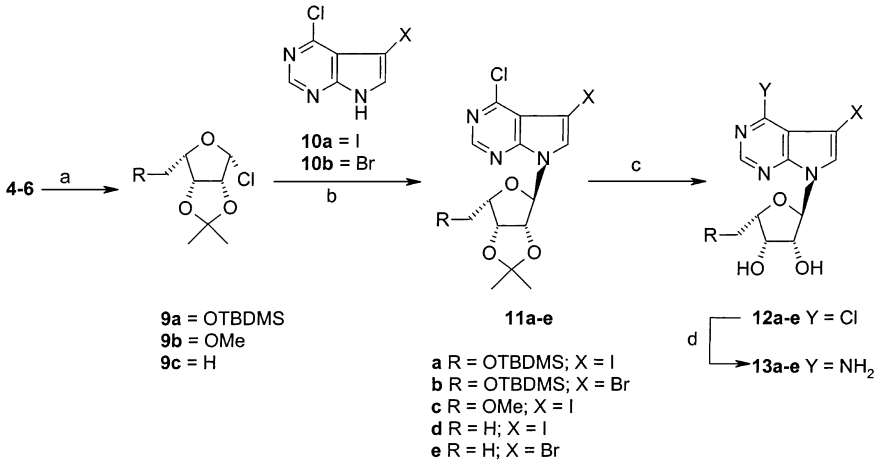
[a] Enzyme inhibition assays were performed on human recombinant AK enzyme. IC_{50} values are results of a single experiment.

Scheme 1[a]



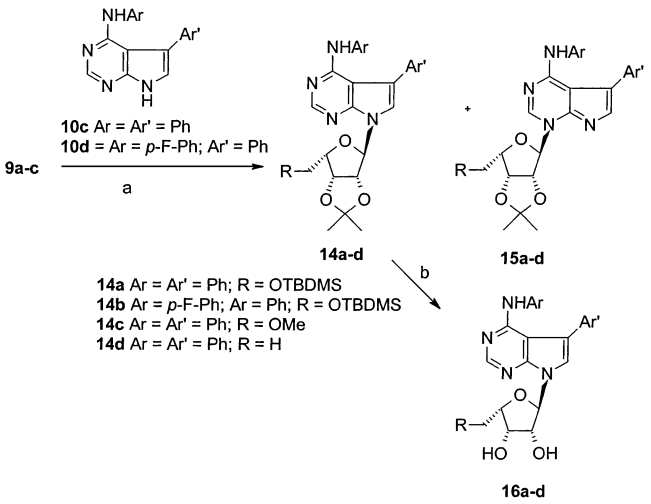
[a] (a) BH_3·THF, NaOH, H_2O; (b) 0.1 N H_2SO_4; (c) 2,2-dimethoxypropane, *p*-TSA; (d) TBDMS-Cl, imidazole, CH_2Cl_2; (e) DMF, NaH, CH_3I; (f) MeOH/HCl; (g) Bz_2O, pyridine; (h) AcOH, Ac_2O/H_2SO_4; (i) H_2/Pd-C.

Scheme 2[a]



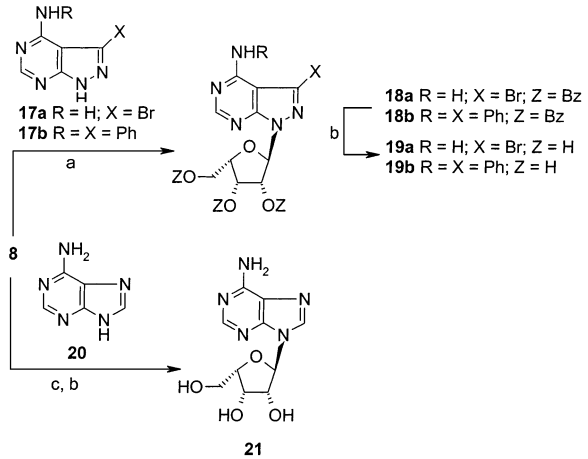
[a] (a) CCl_4, HMPT, toluene; (b) toluene, KOH, TDA-1; (c) 70% aq TFA; (d) MeOH/NH_3.

Scheme 3[a]



[a] (a) Toluene, KOH, TDA-1; (b) 70% aq TFA.

Scheme 4[a]



[a] (a) $MeNO_2/BF_3{\cdot}Et_2O$; (b) MeOH/MeONa; (c) acetonitrile, $SnCl_4$.

the 2- and 3-hydroxyls as described for **4** gave **6** along with 5-deoxy-2,3-*O*-isopropylidene-D-ribose[1] which were separated by silica gel chromatography. Alternatively, **6** could be prepared by tosylation of **3** followed by LAH reduction as described by Townsend et al.[14]

The carbohydrate precursor **8** required for the glycosylation of the pyrazolo[3,4-*d*]pyrimidine and adenine bases was synthesized in three steps starting from L-lyxose. L-Lyxose was first converted to its methyl glycoside and then benzoylated to give **7**, which was subsequently treated with a mixture of acetic anhydride, acetic acid, and sulfuric acid at 0 °C to give **8** in a 47% overall yield.

The lyxofuranosyl chlorides **9a**−**c** used in the glycosylation of pyrrolo[2,3-*d*]pyrimidine bases were generated by reacting **4**−**6** with CCl_4 and hexamethylphosphorus triamide (HMPT) in toluene and were used immediately in the glycosylation step (Scheme 2). The structural assignments of the chlorosugars were based on the ^{1}H NMR spectroscopic data. For example, the anomeric proton of **9a** displayed a doublet at δ 6.2 ppm (J = 4.5 Hz), whereas that of the corresponding α-chloro anomer showed a singlet at δ 6.0 ppm (note that the α and β faces of the L-lyxofuranose ring are opposite to those of the D-ribofuranose ring). The anomeric ratio of the β/α chlorosugars was determined to be 95:5 based on the integrations of the anomeric proton signals. This is consistent with the observations made for α/β ribofuranosyl chlorides.[1,2] The half-life of **9a**, as evidenced by the ^{1}H NMR spectroscopy, was found to be >72 h at ≤ 5 °C in $CDCl_3$ based on the conversion of the β to the α chlorosugar. Similar ^{1}H NMR and stability characteristics were observed for **9b**,**c**.

The solutions of **9a**−**c** were added directly to the stirred mixtures containing the heteroaromatic base **10a**,[15] **10b**,[16] powdered KOH, and TDA-1 in toluene (Scheme 2), and the isolated products were tentatively assigned the structures **11a**−**e**. Acid-catalyzed deprotection followed by treatment with MeOH/NH_3 gave the final compounds **13a**−**e** in 50−55% overall yields.

Glycosylation of 4-*N*-arylamino-5-arylpyrrolo[2,3-*d*]-pyrimidine bases **10c**,**d**[2] by a similar procedure, however, gave two products (Scheme 3). In each case, the major product (65−70% yield) was tentatively assigned the *N*-7-glycosylated structure (**14a**−**d**), whereas the minor product (10−15% yield) was assigned the *N*-1-glycosylated structure (**15a**−**d**). When subjected to deprotection using 70% aqueous trifluoroacetic acid (TFA), **14a**−**d** gave **16a**−**d** in 65−75% yield, whereas **15a**−**d** gave deglycosylated bases **10c**,**d**. A similar

instability of *N*-1-glycosylated tubercidin analogues is well documented.[2]

The structural assignments for the glycosylated products were made by ^{1}H NMR and UV spectroscopic data. Unlike the 2′,3′-*O*-isopropylidene-protected ribofuranosyl nucleosides of the pyrrolopyrimidine bases,[1,2] the anomeric proton of the corresponding L-lyxonucleosides **11a**−**e** and **14a**−**d** displayed an apparent singlet at δ 6.0−6.3 ppm. For the deprotected nucleosides (**12a**−**e**, **13a**−**e**, and **16a**−**d**), the anomeric proton displayed a doublet at δ 6.1−6.3 ppm with a rather large coupling constant (J = 6.8−8.0 Hz vs J = 4−6 Hz for the corresponding ribonucleoside analogues).[1,2] To ascertain the relative stereochemistry of the anomeric proton, efforts were made to synthesize a β-L-lyxonucleoside. To accomplish this, the β-chlorosugar **9c** was heated at 60−65 °C for 3 h, and the resulting α-chlorosugar was subjected to coupling reactions with **10a** and **10c** using the sodium salt, as well as the TDA-1 mediated glycosylation procedures. These reactions, however, failed to provide the desired β-nucleosides, perhaps due to the steric hindrance on the β-face of the α-lyxofuranosyl chloride. Therefore, a definitive proof for the α-configuration was derived from NOE studies on a representative compound. Thus, irradiation of the *endo*-methyl signal of the isopropylidene group of **14c** resulted in an enhancement of the S-1′-H signal at 6.2 ppm indicating that the isopropylidene group and the anomeric proton were on the same side of the furanose ring. Hence the major glycosylation products were assigned the structures **11a**−**e** and **14a**−**d**.

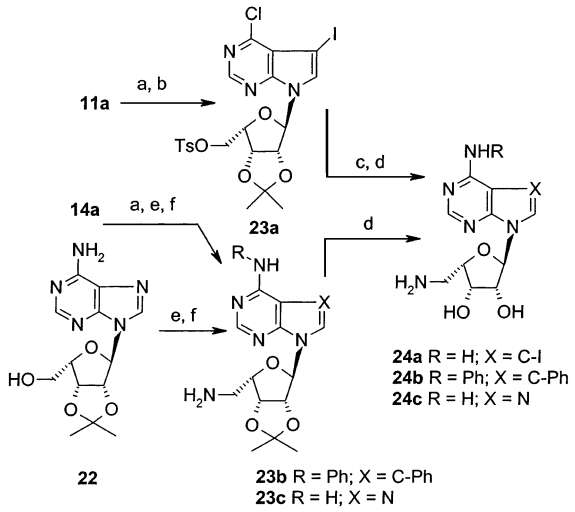
N-1 glycosylated products **15a**−**d** were characterized as described previously[2] by comparing their UV spectroscopic data with those of the *N*-7 glycosylated products.

L-Lyxo-pyrazolo[3,4-*d*]pyrimidine nucleosides **19a**,**b** were prepared as shown in Scheme 4 by glycosylating the fully functionalized heteroaromatic bases **17a**,[17] **17b**[18] with **8** via the BF_3·Et_2O procedure[11] to give **18a** and **18b**, respectively, which were then deprotected using sodium methoxide in methanol to give the final compounds.

The structural assignments of the pyrazolopyrimidine nucleosides were again based on the chemical shifts and the coupling pattern of the anomeric proton signal in the ^{1}H NMR spectrum. Unlike **11a**−**c** or **14a**−**d**, the anomeric proton of the benzoyl protected nucleosides **18a**,**b** displayed a doublet at δ 6.5−6.9 ppm (J = 4.1−4.7 Hz). For the deprotected nucleosides **19a**,**b**, however, the coupling constants were similar to those of the pyrrolopyrimidine nucleosides described above. These coupling constants (J = 6−8 Hz), although larger than those reported for β-D-ribonucleosides of pyrazolo[3,4-*d*]pyrimidine bases,[11] are similar to those reported for L-lyxonucleosides of benzimidazole bases.[14]

The regiochemistry of the glycosylation was established by comparing the UV spectroscopic data of the deprotected nucleosides **19a**,**b** with those of the bases, as well as the *N*-1-ribonucleosides of similar bases.[19,20] For example, the UV spectrum of **19a** showed a λ_{max} at 230 (ϵ 6800), 262 nm (ϵ 5200), and 284 nm (ϵ 7500), whereas **19b** showed a λ_{max} at 224 nm (ϵ 9500), 290 nm (ϵ 17 200) at pH 7 which were similar to the UV spectra of the bases **17a**,**b**. This similarity in UV data of the pyrazolo[3,4-*d*]pyrimidine bases and their nucleosides supported the *N*-1-substitution. A substitution at the *N*-2-position of similar heteroaromatic bases was expected to show a large bathochromic shift in the UV spectrum.[19,20]

Scheme 5[a]



[a] (a) Bu_4NF, THF; (b) DMF, NaH, *p*-toluenesulfonyl chloride (c) MeOH/NH_3: (d) 70% aq TFA; (e) Ph_3P, DIAD, phthalimide (f) EtOH, NH_2NH_2.

9-α-L-Lyxofuranosyladenine **21** was synthesized by coupling adenine (**20**) with **8** using $SnCl_4$ as the Lewis acid, followed by deprotection of the intermediate 9-(2,3,5-tri-*O*-benzoyl-α-L-lyxofuranosyl)adenine with MeOH/MeONa. The melting point of the resulting product was within ±3 °C range of the literature[21] (Scheme 4).

Encouraged by our previous observation[1] that 5′-amino-5′-deoxytubercidin analogues were more potent than their parent nucleosides, a number of 5′-amino-5′-deoxylyxonucleosides were synthesized as follows.

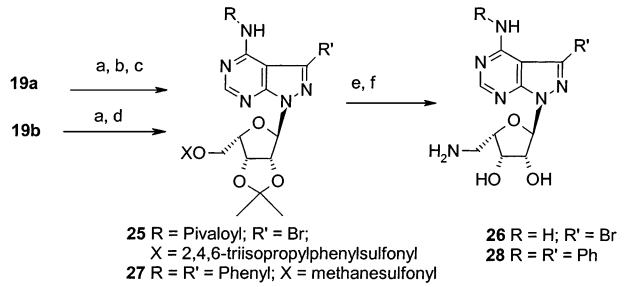
The 5′-amino-5′deoxy pyrrolo[2,3-*d*]pyrimidine lyxonucleosides **24a**,**b** and 5′-amino-5′-deoxy-L-lyxofuranosyladenine **24c** were synthesized starting from the key intermediates **11a**, **14a**, and **22**, respectively, as shown in Scheme 5.

Synthesis of the 5′-amino-5′-deoxy pyrazolo[3,4-*d*]-pyrimidine lyxonucleoside **26** required the use of a bulky trimethylacetyl protecting group to selectively protect the 4-NH_2 of **19a**. Activation of the 5′-hydroxyl was accomplished using 2,4,6-triisopropylbenzenesulfonyl chloride to give **25**. For diaryl nucleoside **19b** the 2′,3′-hydroxyls were first protected with an isopropylidene group, and then the 5′-hydroxyl was activated with methanesulfonyl chloride to give **27**. Amination of **25** and **27** with MeOH−NH_3 followed by deprotection with 70% aq TFA gave **26** and **28** as shown in Scheme 6.

Results

Enzyme Inhibition. IC_{50} values for the AK inhibition were determined as described previously,[1,26] and the results are reported in Table 1. Comparison of the current IC_{50} values with those disclosed in our earlier publications[1,2] indicated that 5-iodo-L-lyxofuranosyltubercidin **13a** (IC_{50} = 68 nM) was somewhat weaker than 5-iodotubercidin **1b**[1] (IC_{50} = 26 nM), whereas the corresponding 5′-deoxy analogue **13d** (IC_{50} = 400 nM)

Scheme 6[a]



[a] (a) Acetone, dimethoxypropane, MeOH/HCl; (b) DIEA, pivaloyl chloride (c) LDA, 2,4,6-triisopropylbenzenesulfonyl chloride; (d) THF, LDA, MsCl; (e) MeOH/NH_3; (f) 70% aq TFA.

Table 2. Antiinflammatory Activity in Rat Carrageenan Paw Edema Model

compd	percent inhibition of paw edema (mean ± SEM) at 10 mg/kg po ($n \geq 6$)
13a	43.8 ± 8.7
16a	47.0 ± 4.6
16b	53.5 ± 5.5
16c	86.0 ± 4.6
16d	90.5 ± 2.5
19b	14.2 ± 4.8

was significantly weaker than its ribo analogue 5′-deoxy-5-iodotubercidin (IC_{50} = 9 nM).[1] A similar trend toward weaker potency of 5-bromo analogues **13b** and **13e** (IC_{50}s = 280 nM and 10 μM, respectively) compared to their ribo-analogues[1] was also observed. 5′-*O*-Me analogue **13c** and its corresponding ribo-analogues were found to be poor inhibitors of AK.

As was observed previously with ribofuranosyl nucleosides,[18,22] 3-Br substituted pyrazolo[3,4-*d*]pyrimidine nucleoside **19a** (IC_{50} = 10 μM) was found to be a weaker AKI than the corresponding pyrrolopyrmidine nucleoside **13b**, whereas the diaryl nucleosides **16a** and **19b** were equipotent to those of the corresponding ribofuranosyl nucleosides.[2,18]

The 5′-amino-5′-deoxy derivatives **24a**, **24b**, and **26** exhibited increased AKI potency compared to their parent nucleosides; however, the increase was not as significant as it was seen with the corresponding 5′-amino derivatives of ribonucleosides.[1] The 5′-amino derivatives of diaryl lyxonucleosides **24b** and **28**, on the other hand, exhibited weaker activity compared to their parent molecules **16a** and **19b**, respectively. This trend toward decreased potency was similar to that observed for the corresponding ribofuranosyl nucleosides.[2,18]

Although L-lyxo-adenosine **21**[21] (IC_{50} = 1.0 μM) was found to be a weak AK inhibitor, it was more potent than adenosine (IC_{50} = 10 μM) as an inhibitor. However, its 5′-amino-5′-deoxy lyxo analogue **24c** (IC_{50} = 10 μM) turned out to be a weaker AKI compared to **21** as well as 5′-amino-5′-deoxy-adenosine (IC_{50} = 120 nM).[23]

Antiinflammatory Activity. Selected potent AKIs were evaluated at 10 mg/kg po in the rat carrageenan paw edema model. Results are shown in Table 2. With the exception of **19b** all the AKIs tested showed moderate to potent inhibition of edema in the rat carrageenan paw model. Dose−responses of selected AKIs in the carrageenan paw edema assay were determined, and the results are shown in Figure 1.

Compound **16a** (GP790) with moderate potency in the carrageenan paw model was chosen to study the dermal

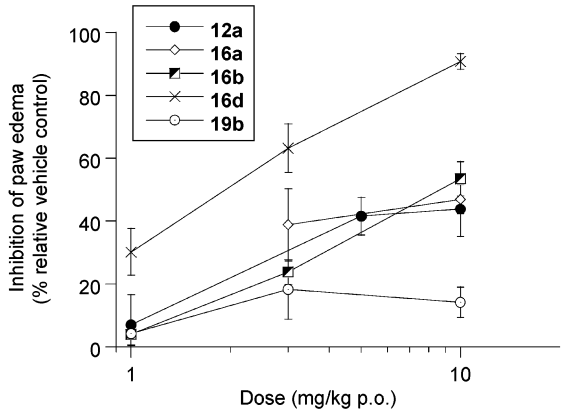


Figure 1. Inhibition of carrageenan-induced paw swelling relative to vehicle control. Data are mean ± SEM from an *n* of at least 6.

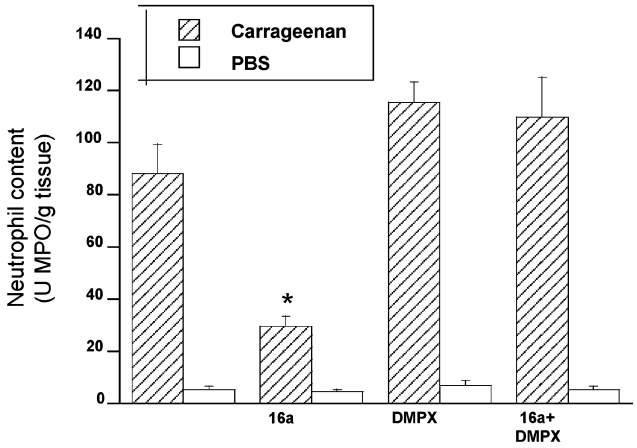


Figure 2. The effect of 16a (GP790) and/or the adenosine A2 antagonist, DMPX, on intradermal neutrophil content following injection of carrageenan. For comparison, neutrophil content following injection with PBS (carrageenan vehicle) is shown. Data are mean ± SEM from an *n* of 14 for each group. An asterisk indicates significant (as assessed with ANOVA and Dunnett's post-hoc test) change in carrageenan-induced neutrophil accumulation from the vehicle/vehicle group.

antiinflammatory effect of AKIs using the rat skin lesion model. After injection of carrageenan, neutrophil infiltration, as reflected by dermal myeloperoxidase (MPO) content, increased approximately 16-fold over that observed after injection of the vehicle as shown in Figure 2. At an oral dose of 10 mg/kg GP790 decreased the neutrophil infiltration by almost 70%. Furthermore, the effect of GP790 was completely reversed in the presence of 3,7-dimethyl-1-propargylxanthine[24] (DMPX), an adenosine A2 receptor antagonist, indicating that adenosine was responsible for the observed antiinflammatory effect. DMPX alone, in the absence of GP790, did not exhibit any effect on MPO content. It is noteworthy that GP790, like other AKIs,[1,2] did not show adenosine agonist activity in the receptors binding assays (data not shown). A significantly more potent compound **16d** in the rat paw edema model was not chosen for the skin lesion test as it caused death in some experimental animals at or near ED_{50} doses.

Discussion

L-Lyxofuranosyl derivatives of tubercidin were synthesized as potential AKIs with reduced propensity to

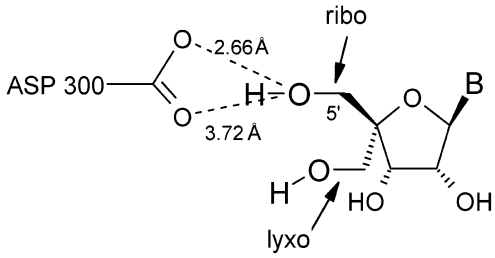


Figure 3.

undergo 5′-phosphorylation and therefore possibly a reduced risk of toxicity. At the outset of our studies we were unsure whether AK would recognize lyxofuranosyl nucleosides since the recent X-ray structure[25] of AK had indicated a strong interaction between the 5′-CH_2OH in the ribo configuration and Asp-300 of the active site. Asp-300 appeared to be outside the range for a similar interaction with the 5′-CH_2OH in the lyxo configuration as shown in Figure 3. This argument was further supported by the absence of substrate activity of L-lyxoadenosine (**21**) toward AK. To our surprise, however, a number of L-lyxo-nucleosides described here showed potent AK inhibition. These results indicated either a slight modification in the active site to align Asp-300 to interact with the 5′-CH_2OH of L-lyxo nucleosides, or the binding interactions of the sugar moiety were provided only by the furanose ring and its 2′ and 3′ hydroxyls. Relatively weaker potencies of **13b**−**e**, **19a**, and **26** in comparison to their corresponding ribofuranosyl analogues indicated variation in binding of the two furanose moieties. Diaryl nucleosides **16a**−**d**, on the other hand, were as potent as their corresponding ribofuranosyl analogues,[2] reiterating our previous argument that a major portion of binding of such nucleosides resulted from aromatic hydrophobic interactions with the backbone of the active site.

Lyxofuranosyladenine **21** (IC_{50} = 1.0 μM) was an inhibitor rather than a substrate of AK because of the unfavorable alignment of its 5′-CH_2OH group for phosphorylation. However, unlike other 5′-amino-5′-deoxy nucleosides, **24c** (IC_{50} = 10 μM) was a weaker inhibitor than its parent nucleoside **21**, which indicated a lack of positive interaction of the 5′-CH_2NH_2 group with Asp-300 of the active site of AK.

Although the pharmacological activities of the compounds disclosed in this article were a function of AK inhibition, the in vivo potencies did not linearly correlate with their IC_{50} values, which can be attributed to potential differences in the pharmacokinetic (PK) properties of the molecules. Reversal of the effect of GP790 in the skin lesion model by an adenosine receptor antagonist clearly indicated that the antiinflammatory effects produced by AKIs was adenosine receptor mediated[8] and is consistent with the elevation of endogenous adenosine levels due to AK inhibition.

Conclusions

The current AKIs were designed to prevent phosphorylation of the 5′-hydroxyl by inverting the stereochemistry at the C4′. While AK readily recognizes certain ribofuranosyl nucleosides, it failed to catalyze the phosphorylation of the corresponding lyxofuranosyl nucleosides. Our studies, however, showed that the lyxofuranosyl moiety is suitable in the design of AK inhibitors. Comparison of the IC_{50} values of L-lyxofuranosyl nucleosides with those of D-ribofuranosyl nucleosides[1,2] suggested that the binding of nucleosides to the active site of AK was largely influenced by the substituents on the base moiety. This was particularly evident in case of diaryl nucleosides, which consistently exhibited potent AKI activity. Therefore, involvement of the sugar, particularly the CH_2OH group, in binding nucleosides to the active site of AK appeared to be relatively minor. The results of biological testing indicated that some of the AKIs exhibit potent antiinflammatory activities in the rat carrageenan-induced paw edema and skin lesion models. Furthermore, reversal of the in vivo activity by an adenosine receptor antagonist suggested that the pharmacological effects of the AKIs was adenosine receptor mediated and was consistent with the elevation of endogenous adenosine levels due to the inhibition of AK. On the basis of the initial test results of the rat paw edema model and dose response tests, several potent compounds were chosen for rat skin lesion tests from which GP790 (**16a**) emerged as a lead compound.

Experimental Section

Proton NMR spectra were recorded on a Varian Gemini-200 spectrophotometer at 200 MHz. NOE experiments were conducted on a Bruker AM-500 spectrophotometer at 500 MHz by NuMega Resonance Laboratories, Inc., San Diego, CA. The chemical shifts are expressed in δ units with respect to tetramethylsilane (δ 0.00) as an internal standard. The UV absorption spectra were recorded on Perkin-Elmer UV/VIS spectrometer and the λ_{min} and λ_{max} are nm units. Melting points are determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography was performed on silica gel GHLF 250 μm plates. Silica gel 230−400 mesh (E. Merck) was used for column chromatography. Elemental analyses were determined by Robertson Microlit Laboratories, Inc. Madison, NJ.

Methyl 2,3-*O*-isopropylidene-L-lyxofuranoside (3). To a stirred solution of methyl 5-deoxy-2,3-*O*-isopropylidene-D-erythropent-4-enofuranoside[13] (**1**, 25.5 g, 137 mmol) in anhydrous THF (300 mL) was added 1 M solution of BH_3:THF (70 mL) over a 30 min period under N_2 atmosphere. A mild exothermic reaction ensued. After 3 h the reaction mixture was cooled in an ice bath, treated carefully with water (30 mL) and 3 M NaOH solution (55 mL). After stirring for 15 min, a 30% solution of H_2O_2 (28 mL) was added to the reaction mixture over 15 min. The resulting clear solution was kept at 55 °C for 30 min, cooled, and extracted with CH_2Cl_2 (3 × 500 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure, and the colorless syrupy residue was kept under high vacuum for 2 h to give **3** (20.0 g, 71%). ^{1}H NMR (DMSO-d_6) δ 1.2 (s, 3H), 1.32 (s, 3H), 3.21 (s, 3H), 3.55 (m, 2H), 3.8 (m, 1H), 4.45 (m, 1H, exchangeable with D_2O), 4.5 (d, J = 4.5 Hz, 1H), 4.72 (m, 1H), 4.81 (s, 1H). This material was identical to the one made starting from the commercial L-lyxose as follows. To a mixture of L-lyxose (10 g, 66.6 mmol), anhydrous $CuSO_4$ (15 g, 94 mmol), 2,2 dimethoxypropane (10 mL, 81 mmol), and methanol (10 mL) was added concentrated H_2SO_4 (0.1 mL). The reaction mixture was stirred at 45 °C for 6 h and filtered, and the acid was neutralized with strongly basic anion resin (Dowex 1 × 2−100 OH^-). The resin was removed by filtration, and the filtrate was evaporated to dryness to furnish **3** as an oil (13.5 g, 37% overall).

5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-L-lyxofuranose (4). Compound **3** (11.2 g, 55 mmol) was suspended in aq 0.1 N H_2SO_4 solution (300 mL) and heated at 85 °C for 4 h. The resulting clear solution was cooled and neutralized with strongly basic anion resin (Dowex 1 × 2−100 OH^-). The solution was filtered, and the filtrate was evaporated under high vacuum. The residue was coevaporated with dry DMF (2

× 20 mL), and the residual syrupy product was kept under high vacuum for 4 h. This material was redissolved in dry DMF (25 mL) to which 2,2-dimethoxypropane (15 mL) and *p*-toluenesulfonic acid (100 mg) were added and stirred at room temperature for 4 h. The volatile portions were evaporated under high vacuum, and the resulting material was purified by chromatography (SiO_2, 10% methanol in CH_2Cl_2) to obtain 2,3-*O*-isopropylidene-L-lyxofuranose (5.88 g, 56%). ^{1}H NMR (DMSO-d_6) δ 1.23 (s, 3H) 1.38 (s, 3H), 3.55 (m, 2H), 4.0 (m, 1H), 4.42 (d, J = 3.8 Hz, 1H) 4.72 (m, 1H), 5.2 (s, 1H), 6.5 (br s, 1H, exchangeable with D_2O).

The above product (5.4 g, 28.4 mmol) and imidazole (4.05 g, 59.6 mmol) were dissolved in CH_2Cl_2 (200 mL), cooled in an ice bath, and treated with a solution of TBDMS-Cl (4.7 g, 31.4 mmol) in CH_2Cl_2 (20 mL) over 15 min. After being stirred for 3 h at room temperature, the reaction mixture was transferred to a separatory funnel and washed with water (2 × 200 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure, and the residue was purified by chromatography (SiO_2, 10% ethyl acetate in hexanes) to give **4** as a colorless viscous liquid (6.8 g, 78%). ^{1}H NMR (DMSO-d_6) δ 0.4−0.95 (m, 15H), 1.21 (s, 3H), 1.32 (s, 3H), 3.75 (m, 2H), 4.0 (m, 1H), 4.4 (d, J = 3.5 Hz, 1H), 4.7 (m, 1H), 5.1 (d, J = 4.1 Hz, 1H), 6.3 (d, J = 4.3 Hz, 1H exchangeable with D_2O).

2,3-*O*-Isopropylidene-5-*O*-methyl-L-lyxofuranose (5). In a 500 mL round-bottom flask NaH (1.6 g of 60% dispersion in oil, 40 mmol) was placed and rinsed with hexanes (2 × 20 mL) under a N_2 atmosphere. Anhydrous DMF (90 mL) was introduced into the flask through a cannula followed by a slow addition of a solution of **3** (5.4 g, 26.4 mmol) in dry DMF (10 mL). After the evolution of gas ceased, a solution of MeI (3 mL, 50 mmol) in dry DMF (5 mL) was added carefully. The reaction mixture was stirred for 4 h, treated with ethanol (5 mL), and evaporated to dryness under high vacuum. The resulting residue was dissolved in ethyl acetate (100 mL) and washed with brine. The organic layer was dried over $MgSO_4$ and evaporated to give a syrupy residue which was purified by chromatography (SiO_2, 10% ethyl acetate in hexanes) to furnish methyl 2,3-*O*-isopropylidene-5-*O*-methyl-L-lyxofuranoside (4.7 g, 81%). ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 3H), 1.43 (s, 3H), 3.3 (s, 3H), 3.4 (s, 3H), 3.65 (m, 2H), 4.15 (m, 1H), 4.52 (d, J = 3.7 Hz, 1H), 4.71 (m, 1H), 4.9 (s, 1H).

The above product was subjected to deprotection with 0.1 N H_2SO_4 solution followed by reprotection with 2,2-dimethoxypropane by the procedure described for **4** to provide **5** as a colorless viscous liquid (2.4 g, 56%). ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 3H), 1.45 (s, 3H), 3.42 (s, 3H), 3.5 (m, 2H), 4.37 (m, 1H), 4.6 (d, J = 3.9 Hz, 1H), 4.8 (m, 1H), 5.4 (s, 1H).

5-Deoxy-2,3-*O*-isopropylidene-L-lyxofuranose (6). To a solution of **2** (8.0 g, 43 mmol) in ethanol (200 mL) was added Pd on C (10%, 800 mg) and hydrogenated in a Parr apparatus at 50 psi of H_2 for 16 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was subjected to the manipulation of the protecting groups as described for **4**. The resulting product was purified by column chromatography (SiO_2, 20% ethyl acetate in hexanes) to give **6** as the major product (4.5 g, 60%). ^{1}H NMR (DMSO-d_6) δ 1.15 (d, J = 6.5 Hz, 1H), 1.25 (s, 3H), 1.36 (s, 3H), 4.1 (m, 1H), 4.4 (d, 1H), 4.58 (m, 1H), 5.05 (d, J = 3.6 Hz, 1H), 6.2 (d, J = 3.8 Hz, 1H, exchangeable with D_2O). A second product (0.5 g, 6.5%) isolated from the column was found to be identical with 5-deoxy-2,3-*O*-isopropylidene-D-ribofuranose[1] by ^{1}H NMR.

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-L-lyxofuranose (8). To a solution of **3** (25 g, 166 mmol) in methanol was added a 2.5 M methanolic-HCl solution (12 mL). The reaction mixture was stirred at room temperature overnight and neutralized with basic resin [Amberlite IRA-400 (OH^-)]. The solvent was evaporated to dryness and the syrupy product was purified by chromatography (SiO_2, 15% methanol in CH_2Cl_2) to provide methyl L-lyxofuranoside (18 g). This material was dissolved in dry pyridine (450 mL), cooled in an ice bath, and treated with benzoic anhydride (168 g, 750 mmol) and 4-*N*,*N*-dimethylaminopyridine (200 mg). After 16 h at room temperature, the reaction mixture was cooled in an ice bath, treated with methanol (25 mL), and evaporated under reduced pressure. The residue was dissolved in ethyl acetate (750 mL) and extracted successively with saturated $NaHCO_3$ solution (2 × 200 mL) and brine (2 × 250 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure, and the residue was purified by chromatography (SiO_2, 35% ethyl acetate in hexane) to furnish methyl 2,3,5-tri-*O*-benzoyl-L-lyxofuranoside as a glassy solid (**7**, 47 g). ^{1}H NMR ($CDCl_3$) δ 3.5 (s, 3H), 4.65 (m, 2H), 4.8 (m, 1H), 5.25 (d, J = 4.4 Hz, 1H), 5.65 (m, 1H), 6.05 (m, 1H), 7.2−8.0 (m, 15H). The product was dissolved in a mixture of glacial acetic acid (200 mL) and acetic anhydride (200 mL). The reaction mixture was cooled to 0−5 °C in an ice bath and treated carefully with concd H_2SO_4 (5 mL). After 16 h the reaction mixture was poured onto crushed ice (500 mL), stirred for 45 min, and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (2 × 200 mL), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography (SiO_2, 20% ethyl acetate in hexanes) gave **8** (38 g, 77% overall from L-lyxose) as a white glassy solid. ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3H), 4.65 (d, J = 3.9 Hz, 2H), 5.0 (m, 1H), 5.7 (m, 1H), 6.15 (m, 1H), 6.4 (s, 1H), 7.25−8.1 (m, 15H).

5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-L-lyxofuranosyl Chloride (9a). A General Method for the Preparation of 9a−c. A solution of **4** (5.5 g, 18 mmol) and CCl_4 (2.3 mL, 23.7 mmol) in toluene (25 mL) was cooled to −10 °C and treated dropwise with hexamethylphosphorus triamide (3.6 mL, 19.8 mmol) over 10 min. After being stirred at 0 °C for an additional 20 min, the reaction mixture was transferred to a separatory funnel and washed quickly with ice cold brine (25 mL). The organic layer was dried ($MgSO_4$) and used directly in the glycosylation step. A small portion of the solution was evaporated at low temperature and dissolved in $CDCl_3$ for the ^{1}H NMR spectrum. δ 0.4−0.95 (m, 15H), 1.22 (s, 3H), 1.32 (s, 3H), 3.78 (m, 2H), 4.0 (m, 1H), 4.14 (m, 1H), 4.35 (m, 1H), 5.7 (d, J = 3.8 Hz, 1H). A singlet of smaller intensity at 6.03 ppm was assigned to the anomeric proton of the α-chloro anomer. Comparison of the anomeric peak integrations indicated the approximate ratio β/α chloro-sugars to be 85:15.

4-Chloro-5-iodo-7-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11a). A General Method for the Preparation of 11a−e. To a stirred mixture of **10a**[15] (3.91 g, 14 mmol), KOH (1.01 g, 18.2 mmol), and TDA-1 (15 mmol) in toluene (60 mL) was added the chloro sugar **9a** generated from **4** (8.51 g, 28 mmol). The reaction mixture was stirred overnight at room temperature and extracted once with brine (50 mL). The organic layer was evaporated, and the residue was purified by chromatography (SiO_2, 20% ethyl acetate in hexane) to give **11a** as an off-white glassy solid (3.56 g, 45%). ^{1}H NMR (DMSO-d_6) δ 0.8 (m, 15H), 1.35 (s, 3H), 1.5 (s, 3H), 3.9 (m, 2H), 4.35 (m, 1H), 5.17 (m, 1H), 5.4 (m, 1H), 6.28 (s, 1H), 8.12 (s, 1H), 8.7 (s, 1H).

5-Bromo-4-chloro-7-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11b). Yield 49%. ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 15H), 1.32 (s, 3H), 1.51 (s, 3H), 3.85 (m, 2H), 4.3 (m, 1H), 5.15 (m, 1H), 5.4 (m,1H), 6.28 (s, 1H), 8.1 (s, 1H), 8.7 (s, 1H).

4-Chloro-5-iodo-7-(2,3-*O*-isopropylidene-5-*O*-methyl-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11c). Yield 40%. ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 3H), 1.6 (s, 3H), 3.4 (s, 3H), 3.6−3.8 (m, 2H), 4.55 (m, 1H), 5.18 (m, 1H), 5.52 (m, 1H), 6.0 (s, 1H), 7.4 (s, 1H), 8.6 (s, 1H).

4-Chloro-5-iodo-7-(5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11d). Yield 40%. ^{1}H NMR (DMSO-d_6) δ 1.2 (d, J = 6.6 Hz, 3H), 1.35 (s, 3H), 1.5 (s, 3H), 4.37 (m, 1H), 5.0 (m, 1H), 5.4 (m, 1H), 6.2 (s, 1H), 8.10 (s, 1H) and 8.68 (s, 1H).

5-Bromo-4-chloro-7-(5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11e). Yield 35%. ^{1}H NMR (DMSO-d_6) δ 1.22 (d, J = 6.3 Hz, 3H), 1.35 (s, 3H), 1.52 (s, 3H), 4.35 (m, 1H), 5.25 (m, 1H), 6.21 (s, 1H), 8.13 (s, 1H) and 8.7 (s, 1H).

4-Chloro-5-iodo-7-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (12a): A general method for deprotecting 12a−e. A mixture of **11a** (3.5 g, 6.2 mmol) and 70% TFA (60 mL) was stirred at room temperature for 30 min and evaporated under high vacuum. The residue was coevaporated with water (2 × 20 mL) and treated with 5% $NaHCO_3$ solution (30 mL), and the resulting crude product was collected by filtration and crystallized from boiling ethanol to provide **12a** as fine needles (1.7 g, 67%): mp 219−220 °C. ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 2H), 4.1 (m, 1H), 4.4 (m, 1H), 4.7 (t, 1H, exchangeable with D_2O), 4.85 (m, 1H), 5.22 (d, 1H, exchangeable with D_2O), 5.48 (d, 2H, exchangeable with D_2O), 6.17 (d, J = 7.15 Hz, 1H), 8.31 (s, 1H), 8.7 (s, 1H). Anal. ($C_{11}H_{12}ClIN_3O_4$) C, H, N.

5-Bromo-4-chloro-7-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (12b). Yield 66%: mp 220−221 °C. ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 2H), 4.15 (m, 1H), 4.4 (m, 1H), 4.65 (t, 1H, exchangeable with D_2O), 4.85 (m, 1H), 5.2 (d, 1H, exchangeable with D_2O), 5.45 (d, 1H, exchangeable with D_2O), 6.19 (d, J = 7.05 Hz, 1H), 8.34 (s, 1H), 8.69 (s, 1H). Anal. ($C_{11}H_{12}ClBrN_3O_4$) C, H, N.

4-Chloro-5-iodo-7-(5-*O*-methyl-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (12c). Yield 72%, mp 147−148 °C. ^{1}H NMR (DMSO-d_6) δ 3.25 (s, 3H), 3.6 (m, 2H,), 4.1 (m, 1H), 4.55 (m, 1H), 4.85 (m, 1H), 5.3 (d, 1H, exchangeable with D_2O), 5.5 (d, 1H, exchangeable with D_2O), 6.2 (d, J = 7.35, 1H), 8.35 (s, 1H), 8.7 (s, 1H).

4-Chloro-5-iodo-7-(5-deoxy-α-L-lyxofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (12d). Yield 68%: mp 192−194 °C. ^{1}H NMR (DMSO-d_6) δ 1.2 (d, J = 6.6 Hz, 3H), 3.95 (m, 1H), 4.5 (m, 1H), 4.9 (m, 1H), 5.2 (d, 1H, exchangeable with D_2O), 5.45 (d, 1H, exchangeable with D_2O), 6.18 (d, J = 7.35 Hz,1H), 8.32 (s, 1H), 8.69 (s, 1H). Anal. ($C_{11}H_{12}ClIN_3O_3$) C, H, N.

5-Bromo-4-chloro-7-(5-deoxy-α-L-lyxofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (12e). Yield 59%: ^{1}H NMR (DMSO-d_6) δ 1.2 (d, J = 6.8 Hz, 3H), 3.97 (m, 1H), 4.6 (m, 1H), 4.85 (m, 1H), 5.22 (d, 1H, exchangeable with D_2O), 5.45 (d, 1H, exchangeable with D_2O), 6.19 (d, J = 7.26 Hz, 1H), 8.35 (s, 1H), 8.71 (s, 1H). MS (CI) m/z 364 $(MH)^+$, 366 $(M + 2)^+$, 368 $(M + 4)^+$, 232 $(base)^+$, 234 $(base + 2)^+$. Anal. ($C_{11}H_{12}ClBrN_3O_3$) C, H, N.

4-Amino-5-iodo-7-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (13a): General Method for the Preparation of 13a−e. A mixture of **12a** (1.6 g, 3.9 mmol) and MeOH−NH_3 (saturated at −10 °C) was heated in a steel bomb for 12 h at 100−120 °C. The bomb was cooled and opened carefully, and the ammonia was allowed to evaporate. The residue was washed with ether (2 × 20 mL), and the resulting product was crystallized from boiling ethanol to give **13a** (1.07 g, 70%): mp 236−238 °C; ^{1}H NMR (DMSO-d_6) δ 3.55 (m, 2H), 4.1 (m, 1H), 4.4 (m, 1H), 4.6 (t, 1H, exchangeable with D_2O), 4.77 (m, 1H), 5.05 (d, 1H, exchangeable with D_2O), 5.4 (1H, exchangeable with D_2O), 6.06 (d, J = 6.95 Hz, 1H), 6.68 (br s, 2H, exchangeable with D_2O), 7.75 (s, 1H), 8.1 (s, 1H). Anal. ($C_{11}H_{13}IN_4O_4$) C, H, N. Following are the yields, melting points, and ^{1}H NMR data for **13b−e** prepared by this procedure on 3−5 mmol scales.

4-Amino-5-bromo-7-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (13b). Yield 69%: mp 245−246 °C; ^{1}H NMR (DMSO-d_6) δ 3.55 (m, 2H), 4.12 (m, 1H), 4.35 (m, 1H), 4.62 (t, 1H, exchangeable with D_2O), 4.7 (m, 1H), 5.1 (d, 1H, exchangeable with D_2O), 5.38 (d, 1H, exchangeable with D_2O), 6.04 (d, J = 6.8 Hz, 1H), 6.65−7.0 (br s, 2H, exchangeable with D_2O), 7.76 (s, 1H), 8.0 (s, 1H). Anal. ($C_{11}H_{13}BrN_4O_4$) C, H, N.

4-Amino-5-iodo-7-(5-*O*-methyl-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (13c). Yield 50%: mp 218−219 °C; ^{1}H NMR (DMSO-d_6) δ 3.21 (s, 3H), 3.5 (m, 2H), 4.08 (m, 1H), 4.26 (m, 1H), 4.75 (m, 1H), 5.15 (d, 1H, exchangeable with D_2O), 5.4 (d, 1H, exchangeable with D_2O), 6.05 (d, J = 7.05 Hz, 1H), 6.6−6.8 (br s, 2H, exchangeable with D_2O), 7.78 (s, 1H), 8.11 (s, 1H). MS (CI) m/z 407 $(MH)^+$, 261 $(base + H)^+$. Anal. ($C_{12}H_{15}IN_4O_4$) C, H, N.

4-Amino-5-iodo-7-(5-deoxy-α-L-lyxofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (13d). Yield 52%: mp 225−227 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (d, J = 6.8 Hz, 3H), 3.9 (m, 1H), 4.23 (m, 1H), 4.8 (m 1H), 5.1 (d, 1H, exchangeable with D_2O), 5.35 (d, 1H, exchangeable with D_2O), 6.0 (d, J = 7.05 Hz, 1H), 6.7 (br s, 2H, exchangeable with D_2O), 7.72 (s, 1H), 8.11 (s, 1H). MS (CI) m/z 377 $(MH)^+$, 261 $(base+H)^+$. Anal. ($C_{11}H_{13}IN_4O_3$) C, H, N.

4-Amino-5-bromo-7-(5-deoxy-α-L-lyxofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (13e). Yield 60%: mp 219−222 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (d, J = 6.8 Hz, 3H), 3.88 (m, 1H), 4.46 (m, 1H), 4.75 (m, 1H), 5.1 (d, 1H, exchangeable with D_2O), 5.4 (d, 1H, exchangeable with D_2O), 5.97 (d, J = 7.15 Hz, 1H), 6.6−6.8 (br s, 2H, exchangeable with D_2O), 7.70 (s, 1H), 7.93 (s, 1H). Anal. ($C_{11}H_{13}BrN_4O_3$) C, H, N.

5-Phenyl-4-*N*-phenylamino-7-α-L-lyxofuranosylpyrrolo-[2,3-*d*]pyrimidine (16a). The heterocycle **10c**[2] (2.86 g, 10 mmol) was glycosylated with **9a** (generated from 6.08 g of **4a**, 20 mmol) by the procedure described for **11a** to provide the intermediate 5-phenyl-4-*N*-phenylamino-7-(2,3-*O*-isopropylidene-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (**14a**) (3.54 g, 62%): UV (methanol) λ_{max} 230 nm (ϵ 5,800), 297 nm (ϵ 18,500). ^{1}H NMR ($CDCl_3$) δ 0.07−0.8 (m 15H), 1.41 (s, 3H), 1.57 (s, 3H), 3.9 (m, 2H) 4.75 (m, 1H), 5.18 (m, 1H), 5.61 (m, 1H), 6.05 (s, 1H), 6.8−7.85 (m, 12H), 8.47 (s, 1H). This intermediate was deprotected by using 70% TFA as described for **12a**, and the resulting product was crystallized from boiling ethanol to furnish **16a** as needles (1.9 g, 74%): mp 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 2H), 4.27 (m, 1H), 4.4 (m, 1H), 4.65 (t, 1H, exchangeable with D_2O), 4.9 (m, 1H), 5.11 (m, 1H, exchangeable with D_2O), 5.42 (m, 1H, exchangeable with D_2O), 6.19 (d, J = 6.85 Hz, 1H), 6.95−7.8 (m, 11H), 8.4 (s, 1H). MS (CI) m/z 419 $(MH)^+$, 287 $(base + H)^+$. Anal.-($C_{23}H_{22}N_4O_4$) C, H, N.

A second product **15a** was isolated from the column as a glassy solid (0.85 g, 15%). UV (methanol) λ_{max} 233 nm (ϵ 6500), 304 nm (ϵ 19 000). ^{1}H NMR ($CDCl_3$) δ 0.06−0.85 (m 15H), 1.48 (s, 3H), 1.66 (s, 3H), 3.8 (m, 2H) 4.75 (m, 1H), 5.18 (m, 1H), 5.61 (m, 1H), 6.15 (s, 1H), 6.8−7.9 (m, 12H), 8.47 (s, 1H).

4-*N*-(4-Fluorophenyl)amino-5-phenyl-7-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (16b). The heterocycle **10d**[2] (3.0 g, 10 mmol) was glycosylated using **9a** (generated from 6.08 g of **4a**, 20 mmol) by the procedure described for **11a** to furnish **14b** (3.5 g, 60%). ^{1}H NMR (DMSO-d_6) δ 0.065−0.9 (m, 15H), 1.35 (s, 3H), 1.52 (s, 3H), 3.9 (m, 2H), 4.4 (m, 1H), 5.2 (m, 1H), 5.45 (m, 1H), 6.2 (s, 1H), 7.1−7.7 (m, 11H), 8.4 (s, 1H). Deprotection of **14b** with 70% TFA by the procedure described for **12a** and crystallization from boiling ethanol provided **16b** in an overall 50% yield: mp 203−204 °C; ^{1}H NMR (DMSO-d_6) δ 3.3 (m, 2H), 4.25 (m, 1H), 4.35 (m, 1H), 4.72 (t, 1H, exchangeable with D_2O), 4.9 (m, 1H), 5.0 (m, 1H, exchangeable with D_2O), 5.4 (m, 1H, exchangeable with D_2O), 6.2 (d, J = 7.18 Hz, 1H), 7.1−7.82 (m, 11H), 8.39 (s, 1H). MS (CI) m/z 437 $(MH)^+$, 305 $(base + H)^+$. Anal. ($C_{23}H_{21}FN_4O_4$) C, H, N.

5-Phenyl-4-*N*-phenylamino-7-(5-*O*-methyl-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (16c). The heterocycle **10c** (2.86 g; 10 mmol) was glycosylated using **9b** (20 mmol) by the procedure described for **11a** to give **14c**, which was deprotected with 70% TFA. The crude product was crystallized from boiling ethanol to give **16c** in an overall 48% yield: mp 162−164 °C; ^{1}H NMR (DMSO-d_6) δ 3.25 (s, 3H), 3.55 (m, 2H), 4.15 (m, 1H), 4.6 (m, 1H), 4.95 (m, 1H), 5.2 (m, 1H, exchangeable with D_2O), 5.45 (m, 1H, exchangeable with D_2O), 6.21 (d, J = 7.07 Hz, 1H), 6.95−7.82 (m, 12H), 8.42 (s, 1H). MS (CI) m/z 433 $(MH)^+$, 287 $(base + H)^+$. Anal. ($C_{24}H_{24}N_4O_4$) C, H, N.

5-Phenyl-4-*N*-phenylamino-7-(5-deoxy-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (16d). The heterocycle **10c** (3.43 g, 12 mmol) was glycosylated using **9c** (generated from **6**, 4.15 g, 24 mmol) by the procedure described for **11a** to provide the intermediate **14d** (3.65, 69%). ^{1}H NMR (DMSO-d_6) δ 1.2 (d, J = 6.9 Hz, 3H), 1.35 (s, 3H), 1.53 (s, 3H), 4.45 (m, 1H), 5.05 (m, 1H), 5.45 (m, 1H), 6.23 (s, 1H), 6.95−7.7 (m, 12H), 9.42(s, 1H). Deprotection of **14d** with 70% TFA followed by crystallization from boiling ethanol gave **16d** (2.55 g, 53% overall): mp 224−225 °C; ^{1}H NMR (DMSO-d_6) δ 1.2 (d, J = 3.9 Hz, 3H), 4.0 (m, 1H), 4.6 (m, 1H), 4.92 (m, 1H), 5.1 (d, 1H,

exchangeable with D_2O), 5.4 (d, 1H, exchangeable with D_2O), 6.18 (d, $J = 7.25$ Hz, 1H), 6.98−7.8 (m, 12H), 8.4 (s, 1H). Anal. ($C_{23}H_{22}N_4O_3$) C, H, N.

4-Amino-3-bromo-1-α-L-lyxofuranosylpyrazolo[3,4-*d*]-pyrimidine (19a). A mixture of 4-amino-3-bromopyrazolo[3,4-*d*]pyrimidine **17a**[17](567 mg, 2.83 mmol), **8** (2.0 g, 3.97 mmol), and nitromethane (15 mL) was heated to gentle reflux and treated with BF_3·etherate (0.5 mL, 4.0 mmol) in one portion. The resulting mixture was refluxed for 2 h, cooled, and treated with 1 mL of triethylamine. Volatiles were evaporated under reduced pressure, and the resulting residue was treated carefully with saturated $NaHCO_3$ solution (50 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried ($MgSO_4$) and evaporated, and the residue was purified by chromatography (SiO_2, 15% ethyl acetate in hexane) to provide **18a** (1.1 g, 61%) as a glassy solid. ^{1}H NMR (DMSO-d_6) δ 4.68 (br d, 2H), 5.2 (m, 1H), 6.25 (m, 1H), 6.55 (m, 2H), 6.5 (d, $J = 5.1$ Hz, 1H), 7.3−8.1 (m, 15H), 8.3 (s, 1H). This intermediate was dissolved in methanol (30 mL) and treated with freshly prepared 2 M NaOMe/MeOH solution to adjust the pH to 10. After stirring for 5 h, the pH of the reaction mixture was adjusted to 3 using strongly acidic ion-exchange resin (Amberlite, IR-120 H^+). The resin was removed by filtration and washed with methanol. The filtrate and the washings were combined and evaporated under reduced pressure, and the residue was purified by chromatography (SiO_2, 10% methanol in CH_2Cl_2). The resulting crude product was crystallized from boiling ethanol to provide **19a** (540 mg, 57% overall), mp 129−132 °C. UV (methanol) λ_{max} 230 nm (ϵ 6800), 262 nm (ϵ 5200), 284 (ϵ 7500). ^{1}H NMR (DMSO-d_6) δ 3.6 (m, 2H), 4.2 (m, 2H), 4.63 (t, 1H, exchangeable with D_2O), 4.9 (m, 1H), 5.1 (br s, 1H, exchangeable with D_2O), 5.6 (br s, 1H, exchangeable with D_2O), 6.1 (d, $J = 7.7$ Hz, 1H), 7.0 (br s, 2H, exchangeable with D_2O), 8.25 (s, 1H). MS (CI) m/z 346 $(MH)^+$, 348 $(M + 2)^+$, 214 (base)$^+$, 216 (base + 2)$^+$. Anal. ($C_{10}H_{12}BrN_5O_4$), C, H, N.

3-Phenyl-4-*N*-phenylamino-1-α-L-lyxofuranosylpyrazolo[3,4-*d*]pyrimidine (19b). To a gently refluxing solution of 4-*N*-phenylamino-3-phenylpyrazolo[3,4-*d*]pyrimidine **17b**[18] (0.5 g, 1.7 mmol) and **7** (1.76 g, 3.5 mmol) in nitromethane (10 mL) was added BF_3·etherate (0.45 mL) and continued refluxing for 2 h. The reaction was worked up as described for **18a** to provide 3-phenyl-4-*N*-phenylamino-1-(2,3,5-tri-*O*-benzoyl-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (658 mg, 55%) as an off white glassy solid. ^{1}H NMR (DMSO-d_6) δ 4.7 (br d, 2H), 5.27 (m, 1H), 6.35 (t, 1H), 6.7 (t, 1H, $J = 2.1$ Hz), 6.9 (d, $J = 4.7$ Hz, 1H), 7.05−8.1 (m, 25H), 8.5 (s, 1H, exchangeable with D_2O), 8.56 (s, 1H). This intermediate was deprotected using 2 M MeONa/MeOH solution, and the resulting crude product was crystallized from boiling ethanol to provide **19b** (305 mg, 42%): mp 234−235 °C; UV (methanol) λ_{max} 224 nm (9500), 290 nm (17 200). ^{1}H NMR (DMSO-d_6) δ 3.73 (m, 2H), 4.3 (m, 2H), 4.65 (t, 1H, exchangeable with D_2O), 5.09 (m, 1H), 5.15 (d, 1H, exchangeable with D_2O), 5.45 (d, 1H, exchangeable with D_2O), 6.25 (d, $J = 6.3$ Hz, 1H), 7.0−7.85 (m, 10H), 8.36 (s, 1H, exchangeable with D_2O), 8.49 (s, 1H). Anal. ($C_{22}H_{21}N_5O_4$) C, H, N.

9-α-L-Lyxofuranosyladenine (α-L-Lyxoadenosine, 21). To a stirred suspension of adenine (**20**, 4.42 g, 32.7 mmol) and **8** (15.0 g, 29.7 mmol) in anhydrous acetonitrile (250 mL) was added $SnCl_4$ (3.5 mL of 1 M solution in CH_2Cl_2) over 10 min. After 16 h a saturated solution of $NaHCO_3$ (50 mL) was added slowly, and the resulting mixture was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated. The residue was purified by chromatography (SiO_2, 5% methanol in CH_2Cl_2). The initial fractions containing unreacted sugar were discarded. The fractions containing the product were pooled and evaporated to provide a glassy solid which was characterized as 9-(2,3,5-tri-*O*-benzoyl-α-L-lyxofuranosyl)adenine (8.52 g, 44%) ^{1}H NMR (DMSO-d_6) δ 4.68 (m, 2H), 5.45 (m, 1H), 6.0 (t, 1H), 6.8 (m, 2H), 7.3−8.1 (m, 17H), 8.22 (s, 1H), 8.45 (s, 1H). This intermediate was deprotected with 2 M NaOMe/MeOH solution to provide **21** (2.5 g, 65%) mp 245−247 °C (lit., 246−249 °C).[21] ^{1}H NMR (DMSO-d_6) δ 3.44 (m, 2H), 4.16 (m, 1H), 4.45 (m, 1H), 4.65 (t, 1H, exchangeable with D_2O), 5.05 (m, 1H), 5.1 (d, 1H, exchangeable with D_2O), 5.5 (d, 1H, exchangeable with D_2O), 5.9 (d, $J = 7.2$ Hz, 1H), 7.3 (br s, 2H, exchangeable with D_2O), 8.16 (s, 1H) and 8.39 (s, 1H). Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

4-Amino-5-iodo-7-(5-amino-5-deoxy-α-L-lyxofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (24a). To a solution of **11a** (1.0 g, 1.7 mmol) in dry THF (50 mL) was added tetrabutylammonium fluoride (5 mL of 1M solution in THF) and stirred for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by chromatography (SiO_2, 20% ethyl acetate in hexanes) to obtain 4-chloro-5-iodo-7-(2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (700 mg, 91%) as a glassy solid. ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 3H), 1.47 (s, 3H), 3.6 (m, 2H), 4.25 (m, 1H), 4.74 (t, 1H, exchangeable with D_2O), 5.15 (m 1H), 5.4 (m, 1H), 6.25 (s, 1H), 8.10 (s, 1H) and 8.7 (s, 1H). To an ice-cold solution of this intermediate (700 mg, 1.55 mmol) in THF (20 mL) was added NaH (75 mg, 60% dispersion in oil, 1.87 mmol,) over 5 min. The reaction mixture was stirred for 20 min and then treated with a solution of *p*-toluenesulfonyl chloride (300 mg, 1.57 mmol) in 5 mL of THF. The resulting mixture was stirred overnight at room temperature. An additional 30 mg of *p*-toluenesulfonyl chloride was added and stirred for 4 h. The solvent was evaporated, and the residue dissolved in ethyl acetate (100 mL) and extracted with water (50 mL). The organic layer was dried ($MgSO_4$) and evaporated, and the residue was purified by chromatography (SiO_2, 20% ethyl acetate in hexanes) to give 4-chloro-5-iodo-7-(2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]-pyrimidine **23a** (550 mg, 60%) as a glassy material. ^{1}H NMR (DMSO-d_6) δ 1.3 (s, 3H), 1.42 (s, 3H), 2.4 (s, 3H), 4.2 (m, 2H), 4.36 (m, 1H), 5.2 (m, 1H), 5.4 (d, $J = 3.3$ Hz, 1H), 6.26 (s, 1H), 7.4 (d, $J = 8.0$ Hz, 1H), 7.75 (d, $J = 8.5$ Hz, 1H), 8.08 (s, 1H) and 8.68 (s, 1H). **23a** was dissolved in methanolic ammonia (35 mL), and the solution was heated in a steel bomb overnight at 120 °C. The bomb was cooled and the ammonia was allowed to evaporate. The residue was purified by chromatography (SiO_2, 15% methanol in CH_2Cl_2) to provide 4-amino-5-iodo-7-(5-amino-5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrrolo[2,3-*d*]pyrimidine (300 mg) as a hygroscopic solid. This material was further subjected to deprotection using 70% TFA by the procedure described for **12a**. The resulting product was dissolved in water (25 mL) and filtered through a Celite pad, and the pH of the filtrate was adjusted to 8 with 3 N NaOH solution. The solution was cooled to furnish an off-white solid that was collected by filtration and washed with water. The pH of the filtrate was adjusted again to 8 and the process was repeated one more time to give **24a** (165 mg, 27% overall from **11a**), mp 189−192 °C. ^{1}H NMR (DMSO-d_6) δ 2.75 (m, 2H), 3.35 (br s, 2H, exchangeable with D_2O), 4.1−4.8 (m, 3H), 5.15 (d, 1H, exchangeable with D_2O), 5.35 (d, 1H, exchangeable with D_2O), 6.03 (d, $J = 6.95$ Hz, 1H), 6.6−6.8 (br s, 2H, exchangeable with D_2O), 7.75 (s, 1H), 8.1 (s, 1H). Anal. ($C_{11}H_{14}IN_5O_3$) C, H, N.

5-Phenyl-4-*N*-phenylamino-7-(5-amino-5-deoxy-α-L- lyxofuranosylpyrrolo[2,3-*d*]pyrimidine (24b). The intermediate **14a** (1.0 g, 1.75 mmol) was subjected to the removal of 5′-*O*-TBDMS group as described for **23** to provide 5-phenyl-4-*N*-phenylamino-7-(2,3-*O*-isopropylidene-α-L-lyxofuranosylpyrrolo[2,3-*d*]pyrimidine as a glassy material (633 mg, 79%). ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 3H), 1.47 (s, 3H), 3.6 (m, 2H), 4.35 (m, 1H), 4. 74 (t, 1H, exchangeable with D_2O), 5.15 (m, 1H), 5.4 (m, 1H), 6.08 (s, 1H), 6.9−7.6 (m, 12H), 8.45 (s, 1H). A mixture of this intermediate (467 g, 1.0 mmol), phthalimide (220 mg, 1.5 mmol), and Ph_3P (394 mg, 1.5 mmol) in dry THF (15 mL) was treated with diisopropylazodicarboxylate (0.33 mL, 1.6 mmol). The reaction mixture was stirred for 4 h and then evaporated to dryness. The residue was dissolved in ethanol (10 mL) containing 97% hydrazine (0.3 mL, 9.3 mmol), heated at reflux for 2 h, and cooled. After being cooled to room temperature, the resulting white solid was removed by filtration and washed with ethanol. The combined filtrate and the

washings were evaporated to give 5-phenyl-4-*N*-phenylamino-7-(5-amino-5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)-pyrrolo[2,3-*d*]pyrimidine **23b** (360 mg). ^{1}H NMR (DMSO-d_6) δ 1.45 (s, 3H), 1.65 (s, 3H), 2.3 (br s, 2H, exchangeable with D_2O), 3.15 (d, J = 8.5 Hz, 2H), 4.5 (m, 1H), 5.26 (m, 1H), 5.65 (m, 1H), 6.08 (s, 1H), 6.9−7.6 (m, 12H), 8.45 (s, 1H). This intermediate was subjected to deprotection by the procedure described for **12a** and crystallization from boiling ethanol to give **24b** (290 mg, 68% overall): mp 125−127 °C. ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 3H, 5′-CH_3), 3.9−4.0 (m, 1H), 4.5−4.65 (m, 1H), 4.85−5.0 (m, 1H), 5.1 (d, J = 4.2 Hz, 1H, exchangeable with D_2O), 5.4 (d, J = 4.5 Hz, 1H, exchangeable with D_2O), 6.19 (d, J = 7.3 Hz, 1H), 6.9−7.8 (m, 12H), 8.41 (s, 1H). Anal. ($C_{23}H_{23}N_5O_3$) C, H, N.

9-(2,3-*O*-Isopropylidene-α-L-lyxofuranosyl)adenine (22). A mixture of **21** (2.4 g, 8.9 mmol), 2,2-dimethoxypropane (4.67 g, 45 mmol), acetone (80 mL), and 5 M methanolic-HCl solution (0.2 mL) was stirred at room temperature overnight. Solid Na_2CO_3 was added, and the solution was filtered. The filtrate was concentrated, and the residue was purified by chromatography (SiO_2, 5% methanol in CH_2Cl_2) to give **22** as a solid (1.9 g, 70%): mp; 180−184 °C (lit.[21] 182−184 °C). ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 3H), 1.48 (s, 3H), 3.63 (m, 2H), 4.33 (m, 1H), 5.12 (m, 1H), 5.4 (d, 1H), 6.27 (s, 1H), 8.56 (s, 1H), 8.65 (s, 1H).

9-(5-Amino-5-deoxy-α-L-lyxofuranosyl)adenine (24c). To a stirred mixture of **22** (180 mg, 0.59 mmol), Ph_3P (230 mg, 0.9 mmol), and phthalimide (132 mg, 0.9 mmol) in dry THF (5 mL) was added diisopropylazodicarboxylate (190 mg, 0.94 mmol). After 4 h, the reaction mixture was concentrated, and the residue was dissolved in ethanol (10 mL) containing 96% hydrazine (0.1 mL, excess). The mixture was refluxed for 30 min and cooled to room temperature, and the resulting solid was removed by filtration and washed with ethanol. The combined filtrate and washings were evaporated, and the residue was purified by chromatography (SiO_2, 15% methanol in CH_2Cl_2) to obtain 9-(5-amino-5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)adenine **23c**. This material was stirred with 70% TFA at room temperature for 45 min and concentrated under high vacuum, and the residue was coevaporated with water (10 mL). The resulting semisolid was purified by HPLC (column: C-18; mobile phase: CH_3CN, MeOH, and 0.2 N acetic acid 40:55:5, using detector wavelength 280 nm, t_R = 4.5−5.0 min) to provide 80 mg of an amorphous solid that was further lyophilized from water to provide **24c** (54 mg, 30% overall yield from **22**), mp 165−168 °C. ^{1}H NMR (DMSO-d_6), δ 3.0−3.7 (br s, 2H, exchangeable with D_2O), 4.28 (m, 1H), 4.66 (m, 1H), 5.15 (m, 1H), 5.98 (d, J = 7.1 Hz, 1H), 7.33 (br s, 2H, exchangeable with D_2O), 8.17 (s, 2H), 8.43 (s, 1H). MS (CI) m/z 437 $(MH)^+$, 305 (base + H)$^+$. Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

4-Amino-3-bromo-1-(5-amino-5-deoxy-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (26). A mixture of **19a** (2.75 g, 7.6 mmol), acetone (106 mL), 2,2 dimethoxypropane (25 mL), and 5 M methanolic-HCl (0.2 mL) was stirred at room temperature for 3 h. Evaporation of the solvent under reduced pressure afforded a crude material that was purified by column chromatography (SiO_2, 5% methanol in CH_2Cl_2) to furnish 4-amino-3-bromo-1-(2,3-*O*-isopropylidene-α-L-lyxofuranosylpyrazolo[3,4-*d*]pyrimidine (2.5 g, 82%) as a glassy solid. ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 3H), 1.46 (s, 3H), 3.55 (m, 2H), 4.25 (m, 1H), 4.72−4.85 (t, 1H, exchangeable with D_2O), 5.05 (m, 1H), 5.35 (m, H), 6.21 (s, 1H), and 8.28 (s, 1H). This material was dissolved in dioxane, cooled in an ice bath, and treated sequentially with diisopropylethylamine (2.9 mL) and trimethylacetyl chloride (0.95 mL, 7.7 mmol). After being stirred overnight, the reaction mixture was poured over crushed ice and extracted with EtOAc (2 × 50 mL). The organic layer was dried ($MgSO_4$) and evaporated, and the residue was purified by chromatography (SiO_2, 5% methanol in CH_2Cl_2) to give 3-bromo-4-*N*-(trimethylacetylamino)-1-(2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (1.65 g, 53%). ^{1}H NMR (DMSO-d_6) δ, 1.35 (m, 15H), 3.55 (m, 2H), 4.25 (m, 1H), 4.77 (t, 1H, exchangeable with D_2O), 5.05 (m, 1H), 5.4 (m, 1H), 6.37 (s, 1H), and 8.8 (s, 1H). To a solution of LDA [generated by treating diisopropylamine (0.33 mL, 3.3 mmol) in THF (15 mL) with n-BuLi (2.0 mL of 1.6 M solution in THF) at −78 °C] was added a solution of **25** (1.44 g, 2.9 mmol) in THF (10 mL), followed by 2,4,6-triisopropylbenzenesulfonyl chloride (1.2 g, 4.0 mmol) in THF (5 mL) over 5 min. The reaction mixture was stirred for 18 h at room temperature, then poured over crushed ice and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated, and the residue was purified by chromatography (SiO_2, 10% ethyl acetate in hexanes) to provide 3-bromo-4-*N*-(trimethylacetylamino)-1-(2,3-*O*-isopropylidene-5-*O*-triisopropylbenzenesulfonyl-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine **25** (300 mg). This intermediate was dissolved in methanolic-ammonia, and the solution was heated in a steel bomb at 110 °C for 16 h. The bomb was cooled, and the ammonia was allowed to evaporate. The residue was purified by chromatography (SiO_2, 5% methanol in CH_2Cl_2) to give 4-amino-3-bromo-1-(5-amino-5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (160 mg, 14%). ^{1}H NMR (CD_3OD) δ 1.39 (s, 3H), 1.53 (s, 3H), 2.99 (br d, 2H), 4.31 (m, 1H), 5.1 (m, 1H), 5.4 (m, 1H), 6.35 (s, 1H), 8.24 (s, 1H). This compound was deprotected using 70% TFA by the procedure described for **12a** to provide crude **26** as a hygroscopic solid. The isolated material was dissolved in water (5 mL) and applied to Dowex 50WX8 cation-exchange resin column. The column was washed with deionized water, followed by 1:1 methanol−water (10 mL). After repeating this process several times until the effluent was neutral to pH paper, the column was then eluted with a 1:1 solution of 1 N NH_4OH−methanol. Evaporation of the effluent under reduced pressure at 35 °C afforded a material that was dissolved in water, filtered, and lyophilized to give **26** as a white amorphous solid (46 mg, 32%): mp 219−222 °C; ^{1}H NMR (CD_3OD) δ 2.99 (br d, 2H), 4.35 (m, 1H), 4.7 (m, 1H), 5.1 (m, 1H), 6.3 (d, J = 6.0 Hz, 1H), 8.23 (s, 1H). MS (CI) m/z 345 $(MH)^+$, 347 $(M + 2)^+$, 214 (base)$^+$, 132 (base − 81)$^+$. Anal. ($C_{10}H_{13}BrN_6O_3$) C, H, N.

3-Phenyl-4-*N*-phenylamino-1-(5-amino-5-deoxy-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (28). A mixture of **19b** (170 mg, 0.4 mmol), acetone (10 mL), 2,2,-dimethoxypropane (1 mL), and 5 M methanolic-HCl (0.2 mL) was stirred overnight at room temperature. The acid was neutralized with solid $NaHCO_3$ (200 mg) and filtered. The filtrate was evaporated, and the residue was purified by chromatography (SiO_2, 2% methanol in CH_2Cl_2) to give 3-phenyl-4-*N*-phenylamino-1-(2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine as an off-white solid (162 mg, 88%). ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 3H), 1.50 (s, 3H), 3.65 (m, 2H), 4.4 (m, 1H), 5.1 (m, 1H), 5.45 (m, 1H), 4.7 (t, 1H, exchangeable with D_2O), 6.41 (s, 1H), 7.02−7.9 (m, 10H), 8.42 (s, 1H, exchangeable with D_2O), 8.53 (s, 1H). To solution of LDA [generated by treating diisopropylamine (0.33 mL, 0.33 mmol) in dry THF (15 mL) with n-BuLi (0.2 mL of 1.6 M solution in THF) at −78 °C] was added a solution of the above intermediate (150 mg, 0.32 mmol) in dry THF (7 mL). After being stirred for 15 min, the reaction mixture was treated with a solution of methanesulfonyl chloride (0.03 mL, 0.375 mmol) in THF (2 mL) and stirred for 48 h. The reaction was quenched with ice-cold NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and evaporated, and the residue was purified by chromatography (SiO_2, CH_2Cl_2) to provide 3-phenyl-4-*N*-phenylamino-1-(2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine **27** as a glassy solid (145 mg, 84%). ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 3H), 1.54 (s, 3H), 3.2 (s, 3H), 4.5 (m, 3H), 5.22 (m, 1H), 5.45 (d, 1H), 6.49 (s, 1H), 7.05−7.9 (m, 10H), 8.39 (br s, 1H, exchangeable with D_2O), 8.54 (s, 1H). This material was dissolved in methanolic-ammonia (25 mL) and heated in a steel bomb at 110 °C for 48 h. The bomb was cooled, and the ammonia was allowed to evaporate. The residue was purified by chromatography (SiO_2, 5% methanol in CH_2Cl_2) to give 4-*N*-phenylamino-3-phenyl-1-(5-amino-5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (100 mg). ^{1}H NMR (CD_3OD) 1.41 (s, 3H), 1.56 (s. 3H), 2.95 (m, 2H), 4.44 (m, 1H), 5.1 (m, 1H), 5.54 (d, 1H), 6.5 (s, 1H), 7.0−7.8 (m, 10H), 8.43 (s, 1H). Deprotection of the intermediate using 70% TFA

followed by crystallization from boiling isopropyl alcohol gave **28** as a TFA salt (66 mg, 48% overall), mp 208−210 °C. ^{1}H NMR (DMSO-d_6), δ 3.1 (br d, 2H), 3.1−3.5 (br s, 2H, exchangeable with D_2O), 4.4 (m, 1H), 4.9 (m, 2H), 6.3 (d, J = 5.9 Hz, 1H), 7.0−8.0 (m, 10H), 8.43 (s, 1H, exchangeable with D_2O), 8.53 (s, 1H). Anal. ($C_{22}H_{22}N_6O_3 \cdot CF_3COOH$) C, H, N.

Enzyme Assay. AK activity was measured in a radiochemical assay similar to the procedure of Yamada et al.,[26] with minor modifications as described previously.[1] The results are as shown in Table 1.

In Vivo Inflammation Assays. Carrageenan-induced paw edema studies were performed as described previously.[8] Compounds were administered at the indicated doses by oral gavage using poly(ethylene glycol)-400 (PEG-400, Sigma Chemical Co., St. Louis, MO) as a vehicle. **16a** was further studied in a model of dermal inflammation as described previously,[8] wherein it was administered at 10 mg/kg po 1 h previous to intradermal injections. To assess the contribution of adenosine to inhibition of neutrophil accumulation, the A2a antagonist DMPX[24] (3,7-dimethyl-1-propargyl xanthine, RBI, Natick, MA) was administered twice at 10 mg/kg ip, 90 min before and 90 min after intradermal injections.

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