Effect of a Chemical Modification on the Hydrated Adenosine Intermediate Produced by Adenosine Deaminase and a Model Reaction for a Potential Mechanism of Action of 5-Aminoimidazole Ribonucleotide Carboxylase

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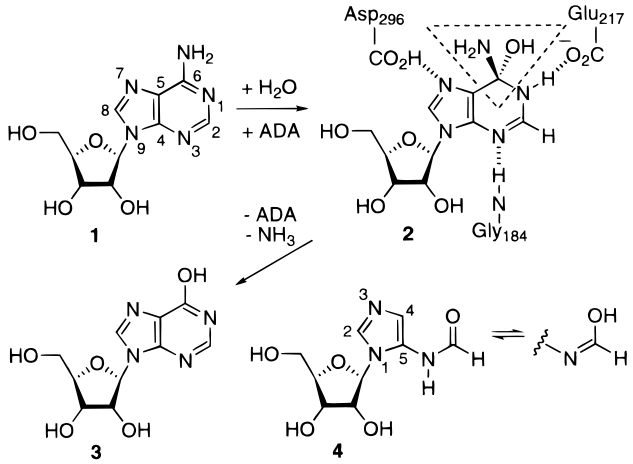
Received May 8, 1997⊗

Using the hydrated adenosine intermediate (6*R*)-6-amino-1,6-dihydro-6-hydroxy-9-(β-D-ribofuranosyl)purine (**2**) produced by adenosine deaminase (ADA, EC 3.5.4.4) as a starting point, the active site probe and inhibitor platform 5-(formylamino)imidazole riboside (FAIRs, **4**) was designed by removal of the −C6(OH)(NH_2)− molecular fragment of **2** generated by the early events of the enzyme-catalyzed hydrolysis. FAIRs was synthesized directly from the sodium salt of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylic acid (CAIR) along a reaction sequence involving a tandem *N*-formylation/decarboxylation that may have a mechanistic connection to the *Escherichia coli pur*E-catalyzed constitutional isomerization of N^5-CAIR to CAIR. The physical and spectral properties of FAIRs were elucidated, its X-ray crystal and NMR solution structures were determined, and its interaction with ADA was investigated. Crystalline FAIRs exists solely as the *Z*-formamide rotamer and exhibits many of the same intramolecular hydrogen bonding events known to contribute to the association of Ado to ADA. In water and various organic solvents, however, FAIRs exists as NMR-distinct, slowly interconverting *Z* and *E* rotamers. This truncated enzymatic tetrahedral intermediate analog was determined to be a competitive inhibitor of ADA with an *apparent* K_i binding constant of 40 μM, a value quite close to that (33 μM) of the natural substrate's K_m. The actual species selected for binding by ADA, though, is likely the minor hydroxyimino prototropic form of *Z*-FAIRs possessing a far lower true K_i value. As the structural features of FAIRs appear well-suited to support its use as a template for constructing active site probes of both ADA and AIR carboxylases, a variety of carbohydrate-protected versions of FAIRs suitable for facile aglycon elaborations were synthesized. The N^3-alkylation, N^3-borane complexation, and C^4-iodination of some of these were investigated in order to assess physicochemical properties that may assist in the elucidation of mechanisms for the AIR carboxylases. The survey of these properties taken together with a reasonable mechanism for the model CAIRs → FAIRs synthetic transformation is interpreted to support a mechanism for the *pur*E-catalyzed N^5-CAIR → CAIR biosynthetic one that involves a carboxylative sp^3-rehybridization of the imidazole C4 atom rather than one possessing a dipole-stabilized C4 sp^2 carbanionic intermediate.

Introduction

Drug development efforts based on modified nucleosides or nucleotides pose some interesting difficulties because of the relative broad specificity of the metabolism of the natural counterparts coupled with the stringent requirements for their biosynthesis. Adenosine deaminase (ADA[1]) catalyzes the essentially irreversible hydrolysis of Ado (**1**) to Ino (**3** in minor lactim form), a key transformation in the cellular processing of the purine nucleosides.[2] An inherited deficiency in this rather nonspecific enzyme is responsible for a severe combined immunodeficiency disease,[3] and its inhibition was once a necessary component of an antitumor chemotherapy regimen.[4] ADA is currently being utilized as a activator of prodrugs of antiviral agents[5] and as an attractive catalyst in organic synthesis[6a] as it is known to operate with reasonable efficiency in aqueous organic media.[6b] As has been supported by crystallographic,[7] computational,[8] and mutagenic[9] analyses, the enzyme-catalyzed process is known to be an $A_N + D_N$ reaction proceeding via Zn-assisted formation of the 1,6-hydrated adenosine (6*R*)-6-amino-1,6-dihydro-6-hydroxy-9-(β-D-ribofuranosyl)-purine (**2**) as an intermediate. In addition to interactions



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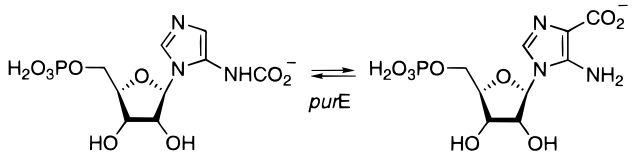
⊗ Abstract published in *Advance ACS Abstracts,* September 15, 1997.

S0022-2623(97)00301-4 CCC: $14.00

with the carbohydrate portion of **1** involved in recognition alone and those in the vicinity of the 6-amino group involved primarily in catalysis, there are ADA active site proton donation events at the N1, N3, and N7 imine nitrogen atoms of **1** that may contribute both to its recognition by the enzyme as well as to its ensuing activation by the same to give **2**.

Design and Rationale

A removal of the tetrahedral intermediate's region of chemical transformation (triangular highlight in **2**) with a preservation of those other regions primarily involved in binding and activation would afford a compound that could be used to probe the importance of the imine nitrogen protonations to binding and the early stages of activation of **1** by ADA. Such a "surgery" performed on **2** involves a removal of the purine C6 ring atom together with its appended hydroxyl and amino groups, a replacement of the purine N1 nitrogen with an oxygen, and an addition of a hydrogen atom to the (former purine) C5 position. An inspection of the structures of the amido and hydroxyimino prototropic forms of (*Z*)-5-(formylamino)imidazole riboside (*Z*-FAIRs) generated in this fashion reveals that they possess a nearly full complement of hydrogen-bond-donating and -accepting sites required by ADA in its association with intermediate **2**. In addition, FAIRs (**4**) bears a striking resemblance to N^5-CAIR (**5**), recently discovered[10a] to be the substrate for the *Escherichia coli pur*E gene product that produces CAIR (**6**) along the AIR carboxylase[10] segment of the *de novo* purine biosynthetic pathway in this organism. While it is now known that this enzyme is required for thiamine biosynthesis via the alternative pyrimidine biosynthetic pathway[11a] and it is suspected that it holds clues to the genomic evolution of bacteria to higher organisms,[11b] details on the mechanism of this apparently unusual biochemical transformation remain obscure.



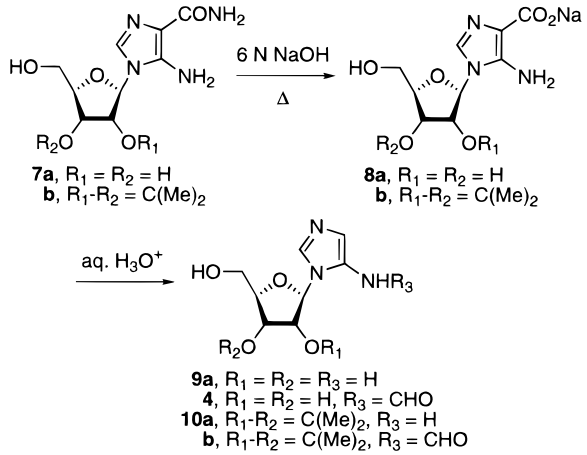
5 **6**

Because of its expected utility in the development of active site and mechanistic probes of both ADA and the AIR carboxylases, we undertook the synthesis of FAIRs and sought to characterize its solution and solid-state structures by a wide variety of methods. Other aims of the present work were to evaluate the effect of FAIRs on the ADA-catalyzed deamination of Ado, to develop good routes to carbohydrate-protected versions of it, and to investigate the reactivities of the 5-(formylamino)-imidazole nucleosides related to certain mechanistic aspects of the AIR carboxylases.

Results and Discussion

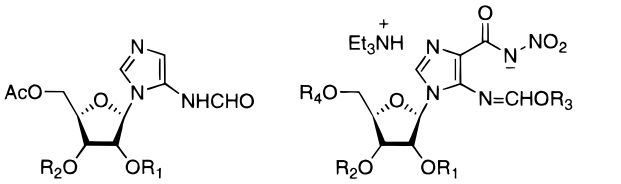
Chemistry. Synthesis of FAIRs in Unprotected and Carbohydrate-Protected Forms. FAIRs itself was prepared in an exceedingly efficient manner (Scheme 1) from the somewhat expensive AICA-riboside (**7a**) according to a modification of a published procedure for the synthesis of AIRs (**9a**).[12] By utilizing a high molarity formate buffer instead of 1 M acetate buffer as the acidic supporting medium for the immediate *in situ* decarboxylation of Na^+ salt **8a** generated[13] from the saponification of **7a** in 6 N NaOH, FAIRs was obtained in a 58% yield in a one-pot fashion from **7a**. The amine **9a** was initially considered to be a candidate intermediate in a tandem decarboxylation/*N*-formylation reaction sequence. It was prepared independently in an improved 90% yield in a one-pot method from **7a** via saponification to **8a** followed directly by decarboxylation in acetate buffer. However, when it was discovered that **9a** did not undergo *N*-formylation at all under these conditions, we had to conclude that decarboxylation does not precede *N*-formylation in the transformation of **8a** to FAIRs. Instead, the C4-carboxylic acid group in **8a** must facilitate an intramolecular *N*-formylation after undergoing mixed anhydride formation with the HCO_2H reagent/solvent.

Scheme 1



Carbohydrate-protected versions desired as intermediates in the synthesis of aglycon-modified FAIRs analogs are not readily synthesized from **4** owing to the competitive reactivity of its formylamino substituent. These instead were prepared from carbohydrate-protected versions of AICARs derivatives. For example, both the 2′,3′-*O*-isopropylidenated version of AIRs (**10a**) and of FAIRs (**10b**) were accessed from 2′,3′-*O*-isopropylidenated AICARs **7b** via decarboxylation of the corresponding CAIRs derivative **8b**. Unlike **9a**, **10a** underwent successful *N*-formylation to give the partially protected FAIRs **11a** upon treatment with $BBr_3/HCO_2H/HCO_2Na$ and the fully protected FAIRs **11b** upon treatment with $B(OH)_3/HCO_2Ac$.[14] To provide for the ready generation of sizable quantities of such compounds, an alternative synthetic route to FAIRs derivatives employing the inexpensive inosine as starting material was developed. In an adaptation of the aminolytic ring opening of protected 1-nitroinosines described by Ariza et al.,[15] we discovered that base-mediated alcoholytic pyrimidine ring opening of these precursors afforded *N*-nitroamidates **12a**−**d** in near-quantitative yield. Saponification and subsequent HCO_2H-supported decarboxylation of one of these (**12a**) afforded FAIRs (49% yield), as did that of its 1-nitroinosine precursor (40% yield).

Physicochemical Analyses of FAIRs. FAIRs was found to exist as an unequal mixture of slowly inter-



11a, $R_1 = R_2 = H$
b, R_1-$R_2 = C(Me)_2$

12a, R_1-$R_2 = C(Me)_2$, $R_3 = Me$, $R_4 = Ac$
b, R_1-$R_2 = C(Me)_2$, $R_3 = Et$, $R_4 = Ac$
c, R_1, R_2, $R_3 = Ac$, $R_3 = Me$
d, R_1, R_2, $R_3 = Ac$, $R_3 = Et$

converting *Z*- and *E*-amide bond rotational isomers in $(CD_3)_2SO$, D_2O, CD_3OD, and C_5D_5N solution, by ^1H and ^{13}C NMR. A dynamic ^1H NMR study[16] conducted using a $(CD_3)_2SO$ solution of **4** revealed these rotamers existing in an equilibrium which, on the basis of a coalescence temperature value (T_c) of 98 °C, has an energy barrier of ~18.5 kcal/mol diagnostic of the interconversion between *Z* and *E* C−N bond rotamers of secondary formamides.[17] MM2 force field computational analysis[18] of appropriate structures gave corroborating results by estimating the major *Z*-FAIRs to be 19 kcal/mol more stable than a 90° C−N bond-rotated intermediary structure and 1.4 kcal/mol more stable than the minor *E*-FAIRs. Phase-sensitive $^1H/^1H$ ROESY (rotating-frame NOE) 2D NMR spectroscopic analysis[19] of **4** in $(CD_3)_2SO$ solution permitted the unequivocal assignment of structures by revealing a diagnostic pattern of a strong ROESY crosspeak with no visible COSY contribution for the NHCHO fragment of the major, *Z* isomer, but a "bleed-through" COSY crosspeak pattern with no visible ROESY contribution for that of the minor, *E* isomer (Figure 1).[20] Finally, the presence of a strong NH/H1′ ROESY crosspeak for *Z*-FAIRs showed it to possess an Ado-like *anti* glycosyl bond rotation conformation in this solution as it does in the solid-state, described next.

An X-ray crystallographic analysis (ORTEP, Figure 2) of FAIRs as it was deposited from an ethanol solution revealed only the *Z* isomer. It displays the ^2E ribofuranose conformation (2′-*endo*, S-type, pseudorotation angle[21] *P* of 165°) with an *anti* aglycon orientation and an interplanar angle between its imidazole ring and formylamino group of ~19°. Examination of the unit cell (Supporting Information) of this crystalline *Z*-FAIRs molecule reveals an extensive 3D intermolecular hydrogen-bonding network that includes N(3)···H−N(5), O(6)···H−O(5′), O(5′)···H−O(2′), and O(2′)···H−O(3′) interactions, many of which involve atoms whose counterparts in Ado undergo hydrogen-bonding with ADA at the active site.

Enzymology. ADA Assay with FAIRs. Evaluation of FAIRs in a standard UV spectrophotometric ADA assay[22,23] revealed this material to be inhibitory in a competitive mode. An *apparent* K_i value of 40 μM quite close to Ado's K_m value of 33 μM[22] was calculated. Interpretation of the significance of this K_i(app) value is far from straightforward, however, and must take into consideration both the identities and concentrations of the various formamide rotamers and prototropes adopted by FAIRs in aqueous solution. The results of many other ADA inhibition studies can be combined to suggest that neither of the amido prototropes found dominant in solution by NMR are bound to ADA. Instead, it is far more likely that the NMR-absent yet inarguably present hydroxyimino prototrope of *Z*-FAIRs is the actual active site-resident species. The Ado analog 1-deazaadenosine (**13**) lacks Ado's N1 imine nitrogen and yet is still such a potent competitive inhibitor of ADA (K_i = 0.18 μM[24]) that it was used in one of this protein's crystal structure determinations.[7a] In this determination, the imine nitrogen at the (former) purine

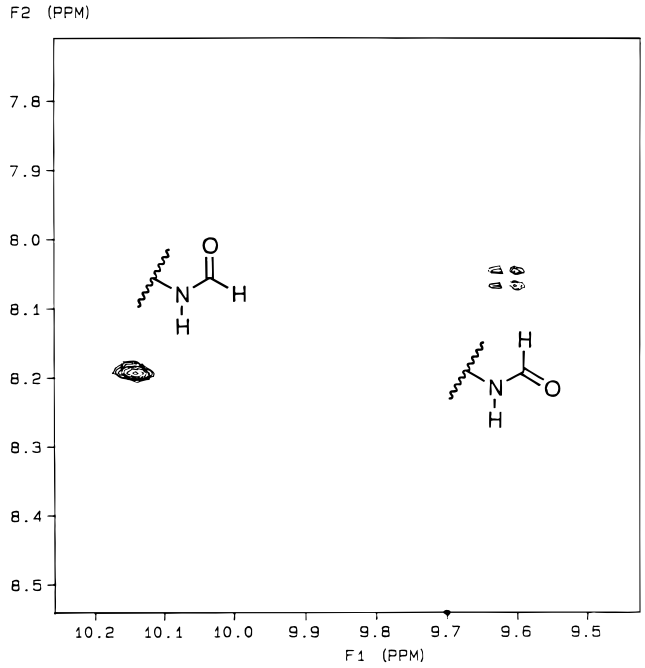


Figure 1. Selected region of a 500 MHz $^1H/^1H$ ROESY 2D NMR spectral plot for FAIRs (**4**) in $(CD_3)_2SO$ solution showing the diagnostic crosspeaks at F1/F2 = δ 10.15/8.22 and 9.62/8.08 ppm due to a strong NOE interaction in the NHCHO fragment of the major, *Z* conformer and a strong *J*-coupling one ("bleed-through" COSY pattern) in that of the minor, *E* conformer, respectively.

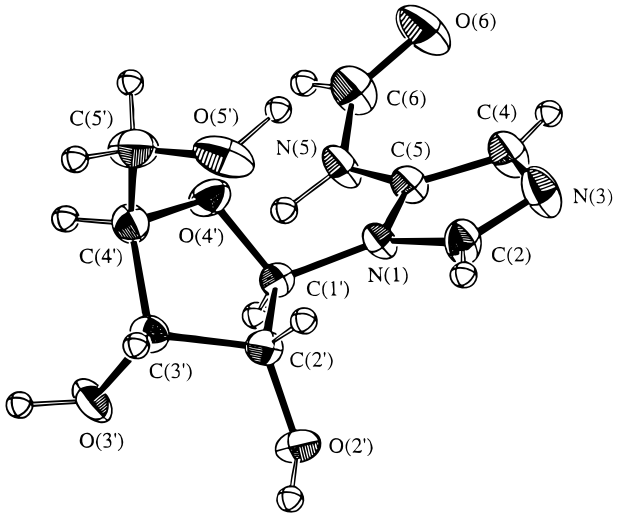
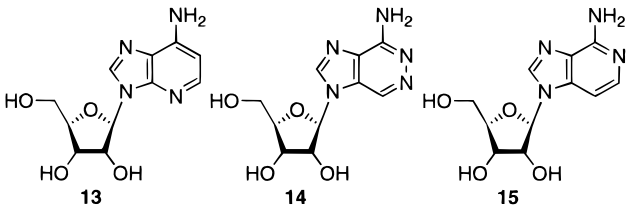
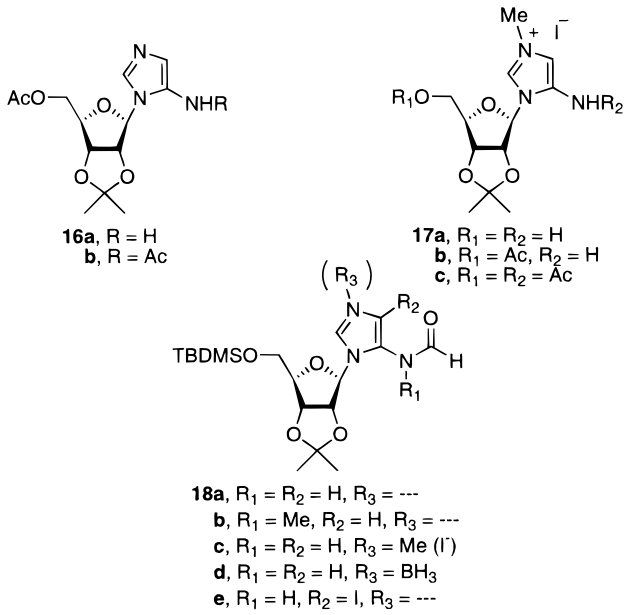


Figure 2. ORTEP drawing and atomic numbering scheme for $C_9H_{13}N_3O_5$ (**4**, FAIRs).



ring 3-position of **13** was found hydrogen-bonded to Gly_{184}, and the CH unit at the (former purine) 1-position had simply displaced the catalytically essential[9b] Glu_{217} residue slightly. By contrast, 2-aza-3-deazaadenosine (**14**), an Ado which retains the 1-position imine nitrogen but lacks the 3-position one, has been shown to be only a poor substrate for and a very weak inhibitor of this enzyme ([**14**] = 100 μM, [Ado] = 25 μM, 15% inhibition).[25] 3-Deazaadenosine (**15**), a nucleoside even closer in structure to Ado than is **14**, has been reported to display *no* ADA substrate or inhibitor activity, even at 100 μM.[26] Thus, the presence of a hydrogen-bond-accepting atom at the purine 3-position is clearly of much greater importance to the ADA/Ado interaction than is that of one at the 1-position. Since the hydroxyimino form of *Z*-FAIRs can accept a hydrogen-bond from Gly_{184} while the amido form cannot,[27] the former is the most likely enzyme-bound species.[28]

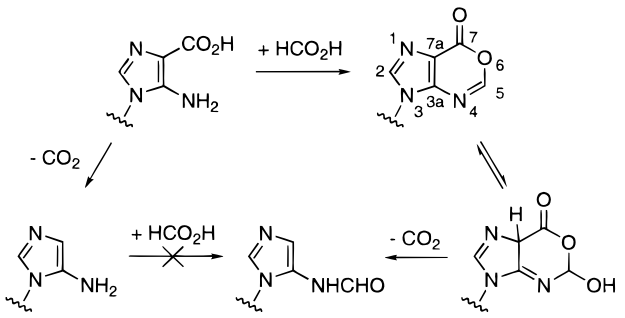
Aglycon-Elaborated AIRs and FAIRs Derivatives. The labilization of the exocyclic bond at the C4 position of 5-aminoimidazoles like that which occurs in the decarboxylation of CAIRs derivatives is highlighted by the facility with which AIRs[12] and derivatives like **16a** acquire a C4 deuterium from D_2O under neutral



conditions. Even though the documented[12] acceleration of this exchange in acid and its attenuation in the corresponding acylaminoimidazoles like **16b** can be taken as indirect evidence for enamine/imine tautomerism as an underlying basis for exchange, the protonation of the basic N3 imine nitrogen to afford a dipole-stabilized C4 sp^2 carbanionic species has not been ruled out thus far. Since this issue has an indirect bearing upon the role of N7 protonation of Ado by ADA and a direct one upon some mechanistic possibilities for the AIR carboxylases, we prepared **16a**,**b**, the 3-methylimidazolium nucleoside salts **17a**−**c**, and the protected FAIRs derivatives **18a**−**e** as models with which to probe the effect of N3 protonation upon C4 deuterium acquisition.

While the H4 of the 5-amine **16a** and corresponding 3-methylimidazolium salts **17a**,**b** were found to exchange slowly in neutral D_2O, neither the acetamide **16b** nor its 3-methylimidazolium salt **17c** did so.

Scheme 2



Likewise, neither the protected FAIRs **18a**, its *N*-formyl-*N*-methylamine derivative (**18b**), its 3-methylimidazolium salt (**18c**), nor its borane complex (**18d**) underwent neutral C4 deuterium acquisition. On the basis of the lack of facile acid-unassisted ring $^1H/^2H$ exchange at C4 of these compounds, it may be safely assumed that *N*3-protonation by itself is not responsible for the acid-catalyzed exchange of H4 in AIRs and that the decarboxylation of CAIRs either in acetate buffer to give AIRs or in formate buffer to give FAIRs does not occur via a dipole-stabilized C4 sp^2-carbanion. Instead, a C4 ring protonation event at some point along the reaction coordinates facilitates loss of CO_2 from an sp^3-hybridized carbon center in a process accompanied by an energetically favorable re-heteroaromatization. The ready C4 iodination of **18a** to give **18e** provides indirect support for the existence of such a transient C4 sp^3-hybridized species. Finally, it may be inferred that the N7-protonation of Ado by the Asp_{296} residue of ADA contributes little to the electrophilic activation of the C6 carbon toward production of **2**.

Relevance of the Tandem *N*-Formylation/Decarboxylation to the *E. coli* AIR Carboxylase. The decarboxylation that takes place in the synthesis of FAIRs from **8a** may be related to that which occurs when the *E. coli pur*E gene product formally translocates the CO_2 group in N^5-CAIR (**5**) to produce CAIR (**6**). A reasonable mechanism for the **8a** → FAIRs chemical transformation is offered in Scheme 2. As mentioned earlier, an initial direct loss of CO_2 from CAIRs is unlikely as AIRs does not undergo *N*-formylation in 95−97% HCO_2H. Instead, the intermediacy of the 5-hydrated imidazo[4,5-*d*][1,3]oxazin-7(6*H*)-one nucleoside 1-deaza-1-oxainosine is indicated. That the reaction coordinate may contain such a hydrate is supported by the observation that efficient production of FAIRs from **8a** is dependent upon the presence of a small (~3−5% v/v) amount of water and by the fact that although stable 5-alkylimidazo[4,5-*d*][1,3]oxazin-7(1*H*)-one nucleosides have been obtained by others via acylation of 5-aminoimidazole-4-carboxylic acids,[13b] our own attempts to access the 5-unsubstituted version by treating **8a** with HCO_2H/Ac_2O or HCO_2Ac consistently promoted facile decarboxylation instead.[29] Dissociation of CO_2 from the oxazinone hydrate to give the hydroxyimino form of FAIRs may be a retro-hetero-Diels−Alder reaction accelerated by N4 protonation and is a model for a potential mechanism of the *E. coli pur*E gene product-catalyzed reaction in the *retro*-biosynthetic direction (i.e., CAIR **6** → N^5-CAIR **5**). By analogy to the microscopic reverse of the decarboxylation shown in Scheme 2, the enzyme in the biosynthetic direction may activate N^5-CAIR to condense with an active-site-bound

CO_2 molecule and produce a 5-hydrated imidazo[4,5-*d*][1,3]oxazine-5,7(4*H*,6*H*)-dione (1-deaza-1-oxaxanthosine) intermediate. Conversion to the 7-hydrate would provide for an isatoic anhydride-like decarboxylation to leave the C5 carbon as CO_2 at a catalytically competent active site as CAIR dissociated from it. This mechanism is consistent with the fact that this *E. coli* enzyme does not utilize either CO_2 or HCO_3^- as a cosubstrate in stoichiometric fashion.[10a] It is of some interest to note that many of the active site features that would be needed to effect this type of transformation are to be found in ADA.

Conclusions

A surgery performed on the structure of the tetrahedral intermediate generated by the hydrolytic enzyme adenosine deaminase has led to the design, synthesis, and enzymatic evaluation of 5-(formylamino)imidazole riboside (FAIRs). It is likely that the hydroxyimino prototrope of the *Z*-FAIRs rotamer is the sole potent competitive inhibitor of ADA, and that with a low K_i binding constant it alone is responsible for the moderate K_i(app) one displayed by the bulk collection of solution structures displayed by FAIRs. The overall structural features of FAIRs render it attractive not only as a template for the pursuit of new potent ADA-inhibitory therapeutic adjuvants but also of mechanistic probes and inhibitors of certain AIR carboxylases. Candidates for such agents now can be readily accessed along one or more of the synthetic routes developed and related herein for the preparation of FAIRs and its derivatives. An investigation of aglycon-modified derivatives of FAIRs already has provided insight into some mechanistic aspects of ADA and an AIR carboxylase.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt capillary apparatus and are uncorrected. Radial preparative-layer chromatography was performed on a Chromatotron instrument (Harrison Research, Inc., Palo Alto, CA) using Merck silica gel-60 PF254 as the adsorbent. Flash chromatography was performed using 230−400 mesh ASTM Merck silica gel-60. Analytical thin-layer chromatography was performed on 250 μm silica gel-GF Uniplates from Analtech, Inc. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 or VXR-500 instrument. These spectra were recorded with tetramethylsilane; 2,2-dimethyl-2-silapentane-5-sulfonic acid, sodium salt (DSS) (δ = 0.0 for ^{1}H); CD_3OD (δ = 49.0 for ^{13}C), or C_5D_5N (δ = 149.9 for ^{13}C), or $(CD_3)_2SO$ (δ = 39.5 for ^{13}C) as internal reference. Compounds **10**, **11**, and **16**−**18** were characterized solely on the basis of their ^{1}H NMR and mass spectral characteristics. The ^{1}H/^{1}H homonuclear shift correlation (COSY) 2D NMR spectrum and short- and long-range ^{1}H/^{13}C heteronuclear shift correlation (HETCOR) 2D NMR spectra of **4** were obtained on the VXR-300 instrument, and the phase-sensitive ^{1}H/^{1}H ROESY 2D NMR spectrum of it was obtained on the VXR-500. FTIR spectra were recorded on a Perkin-Elmer model 1605 spectrophotometer. UV spectrophotometric assays were conducted on an IBM Model 55SX-controlled Shimadzu UV160 UV/Vis spectrophotometer equipped with a Peltier temperature controller and six-cell positioner. Shimadzu's PC-160+ software was used to analyze the raw kinetic data, which was further analyzed in a linear least squares fashion using CricketGraph from Cricket Software. MM2 force field calculations were implemented by Chem-3D Plus, v. 3.0 from Cambridge Scientific Computing, Inc. Lyophilizations were conducted on a Labconco Lypho-Lock 4.5 L benchtop freeze-drier. Formic acid (95−97%) was purchased from the Aldrich Chemical Co. Dowex-50 ion exchange resin, 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside), high-purity adenosine and inosine, Type VIII calf intestinal mucosal adenosine deaminase, and bovine serum albumen were purchased from Sigma Chemical Co. The $(CD_3)_2SO$, $CDCl_3$, D_2O, CD_3OD, and C_5D_5N NMR solvents were obtained from Cambridge Isotope Laboratories, Inc. Elemental microanalyses and mass spectral analyses were obtained from the University of Illinois.

A. Chemical Methods. 5-(Formylamino)-1-(β-D-ribofuranosyl)imidazole (FAIRs, 4). A solution of **7a** (1.01 g, 3.88 mmol) in 4 mL of 6 N NaOH in a flask equipped with an air condenser and a KOH tube (as a CO_2 barrier) was heated on a steam bath (~90 °C) for 20 h with TLC monitoring (30% CH_3OH/CH_2Cl_2 as eluent). The solution was allowed to cool to 23 °C and then was slowly added dropwise with stirring to 20 mL of 95−97% HCO_2H that had been precooled to 0 °C. The resulting solution (pH 1.7) was allowed to warm to 23 °C and then it was degassed via argon perfusion at 23 °C for 24 h (TLC monitoring, same eluent). The solution was adsorbed onto 10 g of silica gel by rotary evaporation in vacuo and this was dried in vacuo overnight before it was loaded onto 80 g of silica gel in a column. Elution first with 30% CH_3OH/CH_2Cl_2 and then with CH_3OH effected removal of product. The eluate was concentrated via rotary evaporation in vacuo, and the wet solid thus obtained was further dried overnight in vacuo. The solid was subjected to repetitive trituration with CH_3CN (total volume *ca.* 1 L), and the combined CH_3CN solutions of **4** were rotary evaporated to dryness, and the residue was dried in vacuo overnight. The crude product was purified by flash chromatography (silica gel, 30% $EtOH/CH_2Cl_2$) to give 543 mg (58%) of **4** as a pale yellow solid. Recrystallization from a highly concentrated CH_3OH solution afforded **4** as a white solid, mp 159−161 °C, of suitable purity for most uses. X-ray quality crystals were grown via slow evaporation of a EtOH/CH_2Cl_2 solution in a desiccator.[30] Nucleoside **4** was found to exist in $(CD_3)_2SO$ solution as a 70:30 *Z*/*E* mixture of rotational forms by NMR. ^{1}H NMR ($(CD_3)_2SO$) δ: 10.15 (s, 1, major), 9.62 (d, J = 10.5 Hz, 1, minor), 8.22 (s, 1, major), 8.08 (d, J = 10.5 Hz, 1, minor), 7.89 (d, J = 0.9 Hz, 1, minor), 7.70 (d, J = 1.1 Hz, 1, major), 6.97 (s, 1, major), 6.86 (s, 1, minor), 5.51 (d, J = 6.2 Hz, 1, major), 5.44 (d, J = 6.0 Hz, 1, minor), 5.41 (t, J = 4.8 Hz, 1, major), 5.40 (d, J = 6.3 Hz, 1, major), 5.37 (d, J = 6.4 Hz, 1, minor), 5.18 (d, J = 4.8 Hz, 1, minor), 5.17 (d, J = 4.5 Hz, 1, major), 5.07 (t, J = 5.4 Hz, 1, minor), 4.23 (pseudo-q, J_{app} = 5.4 Hz, 1, minor), 4.20 (pseudo-q, J_{app} = 6.1 Hz, J_{app} = 5.7 Hz, 1, major), 4.08 (pseudo-q, J_{app} = 5.3 Hz, 1, major), 4.04 (pseudo-q, J_{app} = 4.8 Hz, 1, minor), 3.92 (pseudo-q, Japp = 3.3 Hz, 1, major), 3.86 (pseudo-q, J_{app} = 3.8 Hz, 1, minor), 3.62 (m, 4, both). ^{13}C NMR ($(CD_3)_2SO$) δ: 164.6 (minor), 159.1 (major), 134.8 (minor), 132.8 (major), 126.8 (minor), 126.0 (major), 122.6 (minor), 120.0 (major), 88.1 (major), 87.1 (minor), 85.4 (major), 85.3 (minor), 74.6 (minor), 74.0 (major), 70.4 (major), 70.3 (minor), 61.2 (both). In D_2O solution **4** was a 78:22 *Z*/*E* mixture of rotational forms: ^{1}H NMR (D_2O) δ: 8.33 (s, 1, major), 8.18 (s, 1, minor), 7.94 (s, 1, minor), 7.92 (s, 1, major), 7.07 (d, J = 1.0 Hz, 1, major), 7.05 (d, J = 1.2 Hz, 1, minor), 5.68 (d, J = 5.9 Hz, 1, minor), 5.63 (d, J = 5.4 Hz, 1, major), 4.51 (m, 1, minor), 4.47 (m, 1, major), 4.32 (m, 1, major), 4.30 (m, 1, minor), 4.15 (m, 2, both), 3.82 (m, 4, both. ^{13}C NMR (D_2O) δ: 170.4 (minor), 167.1 (major), 137.8 (minor), 137.3 (major), 126.9 (both), 125.8 (major), 125.6 (minor), 90.4 (major), 90.1 (minor), 87.6 (minor), 87.4 (major), 76.9 (major), 76.8 (minor), 72.8 (minor), 72.7 (major), 63.8 (minor), 63.7 (major). In CD_3OD solution **4** was an 81:19 *Z*/*E* mixture and in C_5D_5N solution a 64:36 *Z*/*E* mixture (selected chemical shift data are given in Table 1 of the Supporting Information). UV, λ_{max} ($\epsilon \times 10^{-3}$): (CH_3OH) 243 (7.4); (pH 1) 218 (5.5); (pH 7) 217 (5.5); (pH 11) 253 (6.4), 219 (5.3). High-resolution FAB (fast atom bombardment) mass spectrum: *m*/*z* 244.0937. $C_9H_{14}N_3O_5$ requires 244.0933. FT-IR (KBr): 1677 cm^{-1} (C=O stretch). Anal. ($C_9H_{13}N_3O_5$) C, H, N. Aqueous pK_{a1} (ImH^+), 4.7; pK_{a2} (NHCHO), >12 (estimated).

Improved Preparation of AIRs (9a). A solution of **7a** (2.50 g, 9.7 mmol) in 20 mL of 6 N NaOH in a polymethylpentene Erlenmeyer flask equipped with a glass tube as air condenser and a KOH tube (as a CO_2 barrier) was heated on a steam bath (~90 °C) for 24 h with periodic TLC monitoring

using 30% CH_3OH/CH_2Cl_2 as eluent. The solution was allowed to cool to 23 °C, and then it was slowly added dropwise with stirring to a solution of 12 mL of glacial AcOH in 100 mL of water. The pH of the resulting solution was adjusted to 4.7 (pH meter) by the addition of AcOH (∼5 mL) and was then degassed via argon perfusion for 18 h with periodic TLC monitoring (same eluent). The aqueous solution was diluted by the addition of 250 mL of water and then it was loaded onto a Dowex-50 (NH_4^+-form) column (250 g). The column was eluted first with water (1.5 L) to pH 7 to remove salts and acids, and then with 1 N NH_4OH (850 mL), followed by 2 N NH_4OH (150 mL). Those fractions containing product by TLC (same eluent) were combined and reduced to less than 50% of its original volume by rotary evaporation in vacuo, and the resulting pH-neutral aqueous solution was lyophilized (48 h) to afford 1.88 g (90%) of **9a** as a gray hygroscopic solid displaying ^{1}H NMR and ^{13}C NMR spectra in $(CD_3)_2SO$ and D_2O solution identical to those previously reported.[12]

5-Amino-1-(2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)-imidazole (iP-AIRs, 10a). A solution of **7b** (3 g, 10.1 mmol) in 20 mL of 6 N NaOH in a 250 mL polymethylpentene Erlenmeyer flask equipped with a KOH tube (as a CO_2 barrier) was heated on a steam bath (∼90 °C) for 24 h with TLC monitoring (30% CH_3OH/CH_2Cl_2 as eluent). The solution was allowed to cool to 23 °C and then it was added dropwise with stirring to a solution of 12 mL of glacial HOAc in 100 mL of distilled water in a flask that had been precooled in an ice bath. The pH of the resulting solution was adjusted (pH paper) to 4.7 by the addition of AcOH (∼5 mL), and it was then allowed to warm to 23 °C. Degassing via argon perfusion was conducted for 24 h with occasional TLC monitoring (30% CH_3OH/CH_2Cl_2 as eluent), and the solution was then diluted by the addition of 250 mL of water and was loaded onto a 250 g Dowex-50 (NH_4^+) column. The column was eluted until pH 6 with 2 L of water to remove salts and acids and then with 1 L of 1 N NH_4OH, followed by 250 mL of 2 N NH_4OH. Fractions found to contain product by TLC were combined and this solution was reduced to less than half of its original volume by rotary evaporation in vacuo at 35−37 °C. The resulting pH-neutral aqueous solution was lyophilized over 48 h to afford **10a** as a gray hygroscopic solid: ^{1}H NMR ($(CD_3)_2SO$) δ: 7.36 (s, 1, H2), 6.07 (s, 1, H4, exchange slowly with D_2O at 23 °C), 5.70 (d, 1, H1′), 5.2 (bs, 1, OH), 5.09 (m, 1, H2′), 4.87 (m, 1, H3′), 4.56 (bs, 2, NH_2), 4.03 (d, 1, H4′), 3.46 (m, 2, CH_2), 1.52 and 1.31 (m, 6, $2CH_3$). ^{13}C NMR ($(CD_3)_2SO$) δ: 137.3 (C2), 130.1 (C5), 113.4 (CMe_2), 110.9 (C4), 88.5 (C1′), 84.7 (C4′), 82.6 (C3′), 80.3 (C2′), 60.9 (C5′), 27.1 (CH_3), 25.3 (CH_3). Upon addition of a catalytic amount of H_2SO_4, the ^{1}H NMR spectrum showed chemical shifts of 8.71, 6.65, and 6.02 ppm for H2, H4, and H1′, respectively, and the H4 resonance exchanged rapidly upon addition of D_2O to the acidified solution. Low-resolution FAB mass spectrum: *m*/*z* 256.1 (MH^+).

5-(Formylamino)-1-(2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)imidazole (iP-FAIRs, 10b). A solution of **7b** (5 g, 16.77 mmol) in 20 mL of 6 N NaOH in a 250 mL polymethylpentene Erlenmeyer flask was heated on a steam bath (∼90 °C) for 24 h. The solution was allowed to cool to 23 °C and then was added dropwise with stirring to a mixture of 75 mL of 97% HCO_2H and 10 mL of 50% NaOH in a flask that had been precooled to −10 °C in a $CaCl_2$/ice bath. The reaction flask was rinsed with 25 mL of additional 97% HCO_2H, and the rinsate was added to the HCO_2H solution. The solution was allowed to warm to 23 °C and then it was degassed via argon perfusion for 24 h. Volatiles were removed in vacuo at 25 °C, and the resulting solid was absorbed onto 25 g of silica gel after it was dissolved in 200 mL of CH_3OH. Purification by column chromatography (10% CH_3OH/CH_2Cl_2 as eluent) afforded 3.07 g (65%) of **10b** existing as a 7:3 mixture of *Z* and *E* rotamers in $(CD_3)_3SO$ solution. ^{1}H NMR ($(CD_3)_3SO$) δ: 10.12 (s, 1, NH major), 9.65 (d, J = 9.8 Hz, 1, NH minor), 8.21 (s, 1, CHO major), 8.07 (d, J = 9.8 Hz, 1, CHO minor), 7.91 (s, 1, H2 minor), 7.79 (s, 1, H2 major), 6.94 (s, 1, H4 major), 6.89 (s, 1, H4 minor), 5.75 (d, J = 2.0 Hz, 1, H1′ major), 5.74 (d, J = 2.0 Hz, 1, H1′ minor), 5.32 (bs, 1, 5′-OH major), 5.18 (bs, 1, 5′-OH minor), 5.08 (m, 1, H2′ minor), 5.06 (dd, J = 3.7, 2.1 Hz, 1, H2′ major), 4.87 (dd, J = 3.8, 1.6 Hz, 1, H3′ major), 4.82 (m, 1, H3′ minor), 4.12 (m, 1, H4′ major), 4.08 (m, 1, H4′ minor), 3.46 (m, 2, 5′-CH_2 major and minor), 1.51 and 1.32 (each s, each 3, $(CH_3)_2C$).

***N*-Formylation of 10a in $BBr_3/HCO_2H/HCO_2Na$: 1-(5-*O*-Acetyl-*β*-D-ribofuranosyl)-5-(formylamino)imidazole (11a).** A solution of **10a** (50 mg, 0.168 mmol) in 1 mL of anhydrous HCO_2H was treated with anhydrous HCO_2Na (55 mg) and the mixture was stirred at 23 °C for 10 min until homogeneous. Then, BBr_3 (16 μL, 0.168 mmol) was added dropwise over 20 min, evoking evolution of a white gas. The reaction mixture was stirred under argon at 23 °C for 18 h until TLC analysis indicated the presence of several polar products. The volatiles were removed in vacuo, and the residue was separated by radial chromatography using 30% CH_3OH/CH_2Cl_2 as eluent to afford 46 mg (50%) of **11a**. ^{1}H NMR ($(CD_3)_2SO$) δ: 10.15 (bs, 1, NH, major, exchanges upon addition of D_2O), 9.70 (bs, 1, NH, minor, exchanges upon addition of D_2O), 8.21 (s, 1, CHO, major), 8.00 (s, 1, CHO, minor), 7.82 (s, 1, H2, minor), 7.69 (s, 1, H2, major), 6.87 (s, 1, H4, major), 6.86 (s, 1, H4, minor), 5.48 (d, J = 4.5 Hz, 1, H1′, major), 5.45 (d, J = 4.5 Hz, 1, H1′, minor), 4.23 (m, 1, H2′ both), 4.18 (m, 1, H3′, both), 4.10 (m, 1, H4′, both), 4.01 (d, 2, CH_2, both), 2.00 (s, 3, CH_3, both). Low-resolution FAB mass spectrum: *m*/*z* 286.1 (MH^+).

***N*-Formylation of 10a in $B(OH)_3/HCO_2Ac$: 1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)-5-(formylamino)-imidazole (11b).** A mixture of **10a** (50 mg, 0.168 mmol) and $B(OH)_3$ (11 mg, 0.168 mmol) in 5 mL of HCO_2Ac was stirred at 0 °C for 5 h, and then was slowly allowed to warm up to 23 °C overnight. The volatiles were removed by rotary evaporation and the residue was separated by radial chromatography using 10% CH_3OH/CH_2Cl_2 as eluent to afford 30 mg (55%) of **11b** as a mixture of *Z* and *E* rotamers: ^{1}H NMR ($(CD_3)_2SO$) δ: 10.10 (bs, 1, NH, major, exchanges upon addition of D_2O), 9.70 (bs, 1, NH, minor, exchanges upon addition of D_2O), 8.23 (s, 1, CHO, major), 8.05 (s, 1, CHO, minor), 7.88 (s, 1, H2, minor), 7.80 (s, 1, H2, major), 6.90 (s, 1, H4, major), 6.76 (s, 1, H4, minor), 5.76 (d, J = 5.4 Hz, 1, H1′, major), 5.70 (d, J = 5.4 Hz, 1, H1′, minor), 5.22 (m, 1, H2′, both), 4.85 (m, 1, H3′, both), 4.25 (m, 1, H4′ both), 4.02 (m, 2, CH_2, both), 2.00 (s, 3, CH_3), 1.48 and 1.25 (each s, each 3, $C(CH_3)_2$). Low-resolution FAB mass spectrum: *m*/*z* 326.1 (MH^+).

Alcoholytic Pyrimidine Ring Opening in 2′,3′,5′-Tri-*O*-protected 1-Nitroinosines. General Procedure. A solution of 150 mg of an appropriately 2′,3′,5′-tri-*O*-protected 1-nitroinosine[15] in 5 mL of CH_2Cl_2 was added dropwise to a solution of excess Et_3N (1.2 mL) and CH_3OH or CH_3CH_2OH (10.5 mL) in CH_2Cl_2 (3 mL) previously equilibrated at −15 °C for 1 h. The reaction mixture was allowed to warm slowly to 23 °C and the yellow solution was stirred until TLC analysis failed to detect starting material (40−120 min). Rotary evaporation of volatiles afforded a near-quantitative yield of product as a foamlike solid or gum that was pure save for a trace of Et_3N or ROH not removable in vacuo. The use of exactly 1 equiv of Et_3N in this procedure was equally satisfactory, but it required an extension of reaction time to 24 h.

12a. ^{1}H NMR ($CDCl_3$) δ: 8.82 (s, 1, CH=N), 7.48 (s, 1, H2), 5.89 (d, J = 2.4 Hz, 1, H1′), 5.00 (d of d, 1, H2′), 4.76 (d of d, 1, H3′), 4.42−4.17 (m, 3, H4′ and 5′-CH_2), 3.93 (s, 3, OCH_3), 3.19 (q, J = 7.2 Hz, 6, $(CH_3CH_2)_3N$), 2.03 (s, 3, CH_3CO), 1.58 and 1.35 (each s, each 3, $(CH_3)_2C$), 1.27 (t, J = 7.2 Hz, 9, $(CH_3CH_2)_3N$). ^{13}C NMR ($CDCl_3$) δ: 170.5 ($COCH_3$), 168.3 ($CONNO_2$), 163.0 (CH=N), 139.8 (C4), 130.7 (C2), 123.8 (C5), 114.5 ($(CH_3)_2C$), 90.5 (C1′), 85.3 (C4′), 83.6 (C2′), 80.7 (C3′), 63.8 (C5′), 54.0 (OCH_3) 45.6 ($(CH_3CH_2)_3N$), 27.1 and 25.3 ($(CH_3)_2C$), 20.6 (CH_3CO), 8.6 ($(CH_3CH_2)_3N$). Low-resolution FAB mass spectrum: *m*/*z* 528.6 (MH^+). Anal. ($C_{22}H_{36}N_6O_9 \cdot 1.5CH_3OH$) C, H, N.

12b. ^{1}H NMR ($(CD_3)_2SO$) δ: 8.65 (s, 1, CH=N), 7.67 (s, 1, H2), 5.81 (d, 1, H1′), 5.14 (d of d, 1, H2′), 4.88 (d of d, 1, H3′), 4.32−4.06 (m, 5, H4′, 5′-CH_2, and CH_3CH_2O), 3.06 (q, 6, $(CH_3CH_2)_3N$), 2.01 (s, 3, CH_3CO), 1.51 (s, 3, $(CH_3)_2C$), 1.34−1.30 (m, 6, $(CH_3)_2C$ and CH_3CH_2O), 1.16 (t, 9, $(CH_3CH_2)_3N$. ^{13}C NMR ($(CD_3)_2SO$) δ: 170.5 ($COCH_3$), 167.0 ($CONNO_2$), 161.6 (CH=N), 138.2 (C4), 130.9 (C2), 124.3 (C5), 113.4 ($(CH_3)_2C$), 88.5 (C1′), 83.8 (C4′), 82.6 (C2′), 80.6 (C3′), 63.7 and 62.4 (C5′

and OCH_2CH_3), 45.5 (($CH_3CH_2)_3N$), 27.0 and 25.2 (($CH_3)_2C$), 20.5 (CH_3CO), 14.1 (OCH_2CH_3), 8.8 (($CH_3CH_2)_3N$). Low-resolution FAB mass spectrum: *m/z* 543.3 (MH^+). Anal. ($C_{23}H_{38}N_6O_9 \cdot H_2O$) C, N; H: calcd, 7.19; found, 6.42.

12c. ^1H NMR ($CDCl_3$) δ: 8.80 (s, 1, CH═N), 7.44 (s, 1, H2), 5.85 (d, J = 4.5 Hz, 1, H1′), 5.52 (d of d, 1, H2′), 5.32 (d of d, 1, H3′), 4.41−4.21 (m, 3, H4′ and 5′-CH_2), 3.84 (s, 3, OCH_3), 2.85 (q, J = 7.2 Hz, 6, ($CH_3CH_2)_3N$), 2.02, 2.03, and 2.05 (each s, each 3, each CH_3CO), 1.09 (t, J = 7.2 Hz, 9, ($CH_3CH_2)_3N$. ^{13}C NMR ($CDCl_3$) δ: 169.0, 169.4, and 170.2 (each CH_3CO), 168.1 ($CONNO_2$), 163.1 (CH═N), 139.7 (C4), 130.1 (C2), 124.3 (C5), 86.1 (C1′), 78.9 (C4′), 73.6 (C2′), 69.6 (C3′), 62.6 (C5′), 53.8 (CH_3O), 45.8 (($CH_3CH_2)_3N$), 20.2, 20.3, and 20.6 (each CH_3CO), 9.8 (($CH_3CH_2)_3N$). High-resolution FAB mass spectrum: *m/z* 573.2525 (MH^+). $C_{23}H_{37}N_6O_{11}$ requires 573.2520. Anal. ($C_{23}H_{36}N_6O_{11} \cdot 1.5CH_3OH$) C, N; H: calcd, 6.82; found, 6.22.

12d. ^1H NMR ($CDCl_3$) δ: 8.74 (s, 1, CH═N), 7.43 (s, 1, H2), 5.84 (d, J = 4.5 Hz, 1, H1′), 5.49 (d of d, 1, H2′), 5.31 (d of d, 1, H3′), 4.35−4.20 (m, 5, H4′, 5′-CH_2, and CH_3CH_2O), 3.16 (q, J = 7.2 Hz, 6, ($CH_3CH_2)_3N$), 2.02, 2.03, and 2.05 (each s, each 3, each CH_3CO), 1.31 (t, J = 7.2 Hz, 3, CH_3CH_2O), 1.20 (t, J = 7.2 Hz, 9, ($CH_3CH_2)_3N$. ^{13}C NMR ($CDCl_3$) δ: 169.1, 169.4, and 170.2 (each CH_3CO), 167.9 ($CONNO_2$), 163.0 (CH═N), 140.3 (C4), 129.9 (C2), 124.1 (C5), 85.9 (C1′), 79.0 (C4′), 73.6 (C2′), 69.7 (C3′), 63.1 and 62.7 (C5′ and OCH_2CH_3), 45.6 (($CH_3CH_2)_3N$), 20.3, 20.4, and 20.7 (each CH_3CO), 14.1 (CH_3CH_2O), 8.6 (($CH_3CH_2)_3N$). High-resolution FAB mass spectrum: *m/z* 587.2681 (MH^+). $C_{24}H_{39}N_6O_{11}$ requires 587.2598. Anal. ($C_{24}H_{38}N_6O_{11} \cdot H_2O$) C, H, N.

Acid Hydrolysis of 12c. A solution of **12c** (0.4 g, 0.76 mmol) in 21 mL of distilled water at 0 °C under argon was treated dropwise over 5 min with TFA (175 μL, 2.3 mmol). The hydrolysis mixture was stirred at 23 °C for 50 min, after which time a TLC analysis of the dark yellow solution confirmed the absence of **12c**. The reaction mixture was extracted with CH_2Cl_2, and the combined organic extracts were dried (Na_2SO_4) and rotary evaporated. The residue was separated by radial chromatography using 15% CH_3OH/CH_2Cl_2 as eluent to give 199 mg (68%) of 1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-aminoimidazole-4-(*N*-nitro)carboxamide as a yellow solid. ^1H NMR (($CD_3)_2SO$) δ: 7.52 (s, 1, H2), 6.10 (s, exchanges upon addition of D_2O, 2, NH_2), 5.84 (d, J = 3.3 Hz, 1, H1′), 5.21 (d of d, 1, H2′), 4.91 (d of d, 1, H3′), 4.30 (d of d, 1, H4′), 4.10 (m, 2, 5′-CH_2), 2.01 (s, 3, $COCH_3$), 1.55 and 1.35 (($CH_3)_2C$. ^{13}C NMR (($CD_3)_2SO$) δ: 170.2 ($COCH_3$), 168.8 ($CONNO_2$), 141.7 (C5), 129.4 (C2), 115.6 (C4), 113.8 (($CH_3)_2C$), 87.8 (C1′), 82.7 (C4′), 82.5 (C2′), 80.5 (C3′), 63.6 (C5′), 26.9 and 25.2 (($CH_3)_2C$), 20.6 (CH_3CO). High-resolution FAB mass spectrum: *m/z* 385.1234 (M^+). $C_{14}H_{19}N_5O_8$ requires 385.1234. A hydrolysis using 50% aqueous HCO_2H at 23 °C for 4 h also gave this product, isolated in a 76% yield.

Alternative Synthesis of FAIRs from a 1-Nitroinosine. NMR-pure **12a** freshly prepared from the corresponding *N*-nitroinosine (408 mg, 1.03 mmol) was dissolved in 6 mL of 6 N NaOH in a polymethylpentene flask and was heated on a steam bath (∼90 °C) under argon for 24 h. The solution was allowed to cool to 23 °C and then was cooled to 0 °C before it was added dropwise with stirring to precooled 97% HCO_2H also at 0°C. The resulting solution was allowed to warm slowly to 23 °C and then was degassed via argon perfusion for 24 h. The solution was adsorbed onto silica gel (6 g) and this was loaded onto a silica gel column (45 g) that was eluted first with 45% CH_3OH/CH_2Cl_2 and then with CH_3OH as eluent. Fractions containing product (TLC) were combined and were rotary evaporated to a solid that was dried overnight in vacuo before it was purified by radial chromatography (30% CH_3OH/CH_2Cl_2 as eluent) to give 121 mg (49%) of **4** with NMR spectral properties identical to those related above. The use of 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-1-nitroinosine as a starting material in this procedure also gave **4**, isolated in a 40% yield.

1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-aminoimidazole (16a). A solution of **7b** (1.5 g, 5.03 mmol) in 7.5 mL of 6 N NaOH in a polymethylpentene Erlenmeyer flask was heated on a steam bath overnight. The reaction mixture was cooled to near 0 °C and was rotary evaporated at 23 °C to dryness. A suspension of the resulting yellow solid in 45 mL of anhydrous pyridine was stirred at 0 °C for 30 min, and then 22.5 mL of freshly distilled (P_2O_5) Ac_2O was added dropwise over 40 min at 0 °C. The reaction mixture was stirred at 7−8 °C for an additional 8−9 h until starting material was no longer detected by TLC. Celite (0.8 g) was added and the mixture was stirred for 20 min before it was suction filtered. The pyridine and excess Ac_2O were removed by rotary evaporation in vacuo at 10−15 °C to leave a brown oily residue which was dissolved in 75 mL of 0.25 M acetate buffer (pH 4.75 by pH meter). This solution was degassed via argon perfusion for 3 h at 35−37 °C, and then it was extracted with CH_2Cl_2. Isolated from the combined organic extracts by chromatography on Florisil (10% CH_3OH/CH_2Cl_2 as eluent), **16a** was a yellow foam−solid (0.99 g, 65%). ^1H NMR (($CD_3)_2SO$) δ: 7.37 (s, 1, H2), 6.09 (s, 1, H4, exchanged slowly upon addition of D_2O and quickly once TFA had been added), 5.74 (d, J = 3 Hz, 1, H1′), 5.15 (m, 1, H2′), 4.87 (m, 1, H3′), 4.50 (bs, 2, NH_2), 4.21 (d, 1, H4′), 4.08 (m, 2, CH_2), 2.02 (s, 3, $COCH_3$), 1.52 and 1.32 (each s, each 3, $C(CH_3)_2$). ^{13}C NMR (($CD_3)_2SO$) δ: 170.5 (CO_2), 137.6 (C2), 130.1 (C5), 114.1 ($C(CH_3)_2$), 111.7 (C4), 88.0 (C1′), 83.4 (C4′), 82.4 (C3′), 80.8 (C2′), 64.1 (C5′), 27.1 and 25.3 ($C(CH_3)_2$), 20.9 (CH_3CO_2). Low-resolution FAB mass spectrum: *m/z* 298.1 (MH^+).

5-(Acetylamino)-1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (16b). A solution of **16a** (100 mg, 0.336 mmol) in 1.5 mL of anhydrous HOAc was treated with 300 mg of tetraacetoxydiborate (TADB)[31] and the reaction mixture was heated on a steam bath (∼90 °C) for 24 h. Ice was added to hydrolyze excess TADB, and then volatiles were removed in vacuo. The black residue was separated by radical chromatography using 15% CH_3OH/CH_2Cl_2 as eluent to afford **16b**. ^1H NMR (($CD_3)_2SO$) δ: 10.05 (bs, 1, NH, minor, exchanges with addition of D_2O), 9.70 (bs, 1, NH, major, exchanges with addition of D_2O), 7.80 (s, 1, H2, minor), 7.75 (s, 1, H2, major), 6.90 (s, 1, H4, minor), 6.78 (s, 1, H4, major), 5.75 (d, 1, H1′, major), 5.70 (d, 1, H1′, minor), 5.10 (m, 1, H2′, both), 4.85 (m, 1, H3′, both), 4.25 (m, 1, H4′ both), 4.02 (m, 2, CH_2, both), 2.00 (m, 6, $2CH_3$), 1.48 and 1.25 (each s, each 3, $C(CH_3)_2$). Low-resolution FAB mass spectrum: *m/z* 340.1 (MH^+).

5-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3-methylimidazolium Iodide (17a). A solution of **10a** (120 mg, 0.5 mmol) in 2 mL of DMF was treated with 3 mL of iodomethane and the reaction mixture was stirred at 23 °C for 24 h until TLC analysis showed one new compound. The volatiles were removed in vacuo, and the residue was characterized as **17a** by NMR. ^1H NMR (($CD_3)_2SO$) δ: 8.78 (s, 1, H2), 6.70 (s, 1, H4, exchange with D_2O under acidic condition), 5.97 (d, J = 3.0 Hz, 1, H1′), 5.09 (m, 1, H2′), 4.90 (m, 1, H3′), 4.03 (d, 1, H4′), 3.77 (s, 3, N3-CH_3), 3.46 (m, 2, CH_2), 1.52 and 1.31 (each s, each 3, $C(CH_3)_2$). ^{13}C NMR (($CD_3)_2SO$) δ: 139.5 (C2), 129.6 (C5), 113.7 (CMe_2), 103.5 (C4), 91.2 (C1′), 87.4 (C4′), 83.8 (C3′), 81.1 (C2′), 61.3 (C5′), 34.8 (CH_3), 27.3 (CH_3), 25.5 (CH_3).

1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-amino-3-methylimidazolium Iodide (17b). A mixture of **16a** (40 mg) and 1 mL of freshly distilled CH_3I in 1 mL of anhydrous CH_2Cl_2 was kept at 23 °C overnight, at which point TLC analysis indicated the starting material had been completely consumed and a single product had formed. The volatiles were removed by rotary evaporation, giving 55 mg (90%) of **17b**. ^1H NMR ($CDCl_3$) δ: 9.15 (s, 1, H2), 6.98 (s, 1, H4), 5.86 (d, J = 3 Hz, 1, H1′), 5.40 (m, 1, H2′), 4.95 (m, 1, H3′), 4.75 (bs, 2, NH_2), 4.40 (m, 1, H4′), 4.20 (m, 2, CH_2), 3.89 (s, 3, NCH_3), 2.07 (s, 3, CH_3), 1.57 and 1.36 (each s, each 3, $C(CH_3)_2$). Low-resolution FAB mass spectrum: *m/z* 312.1 (($M - I)^+$).

5-(Acetylamino)-1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3-methylimidazolium Iodide (17c). A solution of **17b** (70 mg, 0.24 mmol) in 1 mL of anhydrous HOAc was treated with 125 mg of TADB, and the mixture was heated at 80 °C overnight. Aqueous CH_3OH was added, and the volatiles were removed in vacuo. The residue was separated by radial chromatography using 20% CH_3OH/CH_2Cl_2 as eluent to afford 25 mg (30%) of **17c** as a mixture of *Z* and *E* rotamers.

^{1}H NMR ($CDCl_3$) δ: 9.65 (bs, 1, NH), 9.01 (s, 1, H2), 7.40 (s, 1, H4), 6.05 (d, 1, H1′), 5.35 (m, 1, H2′), 4.85 (m, 1, H3′), 4.40 (m, 1, H4′), 4.30 (m, 2, CH_2), 3.98 (s, 3, NCH_3), 2.07 (d, 6, $2CH_3$), 1.57 and 1.36 (each s, each 3, $C(CH_3)_2$). Low-resolution FAB mass spectrum: m/z 354.1 ($(M - I)^+$).

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]-5-(formylamino)imidazole (18a). A solution of **10b** (0.65 g, 2.3 mmol) and TBDMS-Cl (0.48 g, 3.2 mmol) in 1.8 mL of anhydrous pyridine was stirred at 23 °C for 24 h. Absolute EtOH (2 mL) was then added, and the reaction mixture was evaporated to dryness in vacuo. The product was separated by column chromatography using 5% CH_3OH/CH_2Cl_2 as eluent, affording 0.86 g (94%) of **18a** as a yellow-brown solid existing as a 1:1 mixture of *Z* and *E* rotamers in CD_3Cl solution. ^{1}H NMR ($CDCl_3$) δ: 8.33 (s, 1, CHO major), 8.29 (bs, exchanges with D_2O, 1, NH major), 8.22 (d, J = 11 Hz, 1, CHO minor), 7.67 (d, J = 11 Hz, exchanges with D_2O, 1, NH minor), 7.62 (s, 1, H2 major), 7.46 (s, 1, H2 major), 7.28 (s, 1, H4 major), 6.96 (s, 1, H4 minor), 5.72 (d, J = 3.0 Hz, 1, H1′ minor), 5.71 (d, J = 3.0 Hz, 1, H1′, major), 4.94 and 4.88 (m, 1, H2′ major and minor), 4.76 (m, 1, H3′ major and minor), 4.23 (m, 2, H4′ major and minor), 4.0−3.8 (m, 2, 5′-CH_2 major and minor), 1.59 and 1.36 (each s, each 3, $(CH_3)_2C$), 0.91 (s, 9, $(CH_3)_3C$), 0.13 (s, 6, $(CH_3)_2Si$).

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]-5-(*N*-formyl-*N*-methylamino)imidazole (18b). A solution of **18a** (100 mg, 0.23 mmol) in 2 mL of CH_3I was treated all at once with $AgBF_4$ (54 mg, 0.27 mmol), producing a precipitate. The reaction mixture was stirred at 23 °C in the dark for 12 h and then it was suction filtered. The filtrate was evaporated to dryness and separated by radial chromatography using 10% CH_3OH/CH_2Cl_2 as eluent to afford **18b**. ^{1}H NMR ($CDCl_3$) δ: 8.71 (d, J = 1.5 Hz, 1, H2), 8.35 (s, 1, CHO), 7.44 (d, J = 1.5 Hz, 1, H4), 6.00 (d, J = 2.7 Hz, 1, H1′), 4.85 (m, 1, H2′), 4.67 (m, 1, H3′), 4.53 (s, 1, H4′), 3.83 (m, 2, 5′CH_2), 3.86 (s, 3, CH_3), 1.54 and 1.31 (each s, each 3, $(CH_3)_2C$), 0.79 (m, 9, CMe_3), 0.03 (s, 6, $2(SiMe_2)$). ^{13}C NMR ($CDCl_3$) δ: 159.5 (CHO), 130.5 (C2), 125.7 (C5), 116.0 (CMe_2), 114.4 (C4), 94.1 (C1′), 87.4 (C2′), 84.3 (C3′), 80.5 (C4′), 62.4 (C5′), 36.1 (NCH_3), 24.7 (CCH_3), 24.2 ($SiCH_3$), 17.3 (CMe_3).

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]-5-(formylamino)-3-methylimidazolium Iodide (18c). A solution of **18a** (100 mg, 0.23 mmol) in 3 mL of CH_3I was kept at 23 °C under argon for 2.5 h when TLC indicated the absence of starting material and the presence of a new compound. The volatiles were removed by rotary evaporation, affording **18c** as a yellow glassy solid: ^{1}H NMR ($CDCl_3$) δ: 9.80 (s, 1, NH, exchanges upon addition of D_2O), 9.34 (s, 1, CHO), 8.41 (s, 1, H2), 7.52 (s, 1, H4), 6.26 (d, J = 3 Hz, 1, H1′), 4.90 (m, 1, H2′), 4.69 (m, 1, H3′), 3.93 (m, 2, 5′CH_2), 4.00 (s, 3, CH_3), 1.55 and 1.30 (each s, each 3, $(CH_3)_2C$), 0.84 (m, 9, CMe_3), 0.03 (s, 6, $2(SiMe_2)$). ^{13}C NMR ($CDCl_3$) δ: 160.3 (CHO), 131.7 (C2), 126.5 (C5), 117.0 (CMe_2), 114.0 (C4), 95.2 (C1′), 88.4 (C2′), 85.4 (C3′), 81.3 (C4′), 63.7 (C5′), 37.6 (NCH_3), 25.8 (CCH_3), 25.3 ($SiCH_3$), 18.2 (CMe_3). Low-resolution FAB mass spectrum: m/z 412.2 ($(M - I)^+$).

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]-5-(formylamino)imidazole Borane Complex (18d). A solution of **18a** (100 mg, 0.232 mmol) in 2 mL of THF was treated with 0.53 mL of 1 M BH_3 in THF dropwise, evoking an evolution of gas. The mixture was stirred at 23 °C for 5 h until TLC analysis (5% CH_3OH/CH_2Cl_2) indicated the presence of one new compound. The volatiles were removed in vacuo, and the yellow residue was separated by radial chromatography using CH_2Cl_2 and then 5% CH_3OH/CH_2Cl_2 as eluents, giving 88 mg (80%) of **18d**. ^{1}H NMR ($CDCl_3$) δ: 8.32 (s, 1, CHO, major), 8.20 (s, 1, CHO, minor), 8.08 (s, 1, NH, major), 7.95 (s, 1, NH, minor), 7.76 (s, 1, H2, major), 7.60 (s, 1, H2, minor), 7.33 (s, 1, H4, major), 7.01 (s, 1, H4, minor), 5.67 (d, J = 3.6 Hz, 1, H1′), 4.91 (m, 1, H2′), 4.82 (m, 1, H3′), 4.43 (m, 1, H4′), 3.85 (m, 2, 5′CH_2), 1.57 and 1.35 (each s, each 3, $(CH_3)_2C$), 0.85 (m, 9, CMe_3), 0.12 (s, 6, $2(SiMe_2)$).

1-[5-*O*-(*tert*-Butyldimethylsilyl)-2,3-*O*-isopropylidene-β-D-ribofuranosyl]-5-(formylamino)-4-iodoimidazole (18e). A solution of **18a** (1.74 g, 4.38 mmol) in 10 mL of anhydrous CH_2Cl_2 was treated with K_2CO_3 (1.8 g) and the suspension was stirred at 23 °C for 30 min before a suspension of *N*-iodosuccinimide (0.985 g, 4.38 mmol) in 25 mL of CH_2Cl_2 was added. The reaction mixture was stirred at 23 °C for 30 min, and then the mixture was suction filtered. The filtrate was rotary evaporated to dryness and the residue was purified by column chromatography using 2.5% CH_3OH/CH_2Cl_2 as eluent to afford 1.79 g (78%) of **18e** as a yellow foam: ^{1}H NMR ($CDCl_3$) δ: 8.38 (s, 1, CHO, minor), 8.19 (d, J = 10 Hz, collapses to a singlet upon addition of D_2O, 1, CHO, major), 7.91 (bs, exchanges upon addition of D_2O, 1, NH, minor), 7.75 (s, 1, H2, minor), 7.72 (s, 1, H2, major), 7.62 (d, J = 10 Hz, exchanges upon addition of D_2O, 1, NH, major), 5.68 (d, J = 2 Hz, 1, H1′, minor), 5.73 (d, J = 2 Hz, 1, H1′, major), 4.90 (m, 1, H2′, major), 4.80 (m, 1, H2′, minor), 4.71 (m, 1, H3′, both), 4.25 (m, 1, H4′, both), 3.90 (m, 2, CH_2, both), 1.49 (s, 3, CH_3, major), 1.47 (s, 3, CH_3, minor), 1.27 (s, 3, CH_3, major), 1.26 (s, 3, CH_3, minor), 0.83 (s, 9, $3CH_3$, major), 0.81 (s, 9, $3CH_3$, minor), 0.04 (s, 6, $2CH_3$, major), 0.04 (s, 6, $2CH_3$, minor). ^{13}C NMR ($CDCl_3$) δ: 164.0 (CHO, major), 159.8 (CHO, minor), 136.3 (C2, major), 135.9 (C2, minor), 115.0 (C4, major), 114.0 (C4, minor), 93.5 (C5, major), 93.0 (C5, minor), 91.7 (C1′, minor), 90.9 (C1′, major), 85.5 (C2′, minor), 86.1 (C2′, major), 85.7 (C3′, minor), 85.2 (C3′, major), 80.1 (C4, both), 62.1 (C5′, both), 26.1 (CH_3), 0.0 (CH_3). Low-resolution DCI (direct chemical ionization) mass spectrum: m/z 524 (MH^+).

B. NMR Spectral Methods. ROESY 2D NMR Analysis of FAIRs. A 500 MHz phase-sensitive ^{1}H/^{1}H ROESY 2D NMR spectrum of **4** in $(CD_3)_2SO$ solution (Figure 3 of the Supporting Information) obtained in phase-sensitive mode by the method of Kessler et al.[19] with a mixing time of 500 ms revealed the presence of diagnostic crosspeaks (Figure 1) at F1/F2 = δ 10.15/8.22 and 9.62/8.08 ppm due to a strong NOE interaction in the NHCHO fragment of the major, *Z* conformer and a strong *J*-coupling one ("bleed-through" COSY pattern) in that of the minor, *E* conformer, respectively. For each of the 1024 increment fids, 16 transients of 2048 data points were collected. The spectral window was 5385 Hz, and all of the NOE crosspeaks were negative.

COSY 2D NMR Analysis of FAIRs. A 300 MHz ^{1}H/^{1}H COSY 2D NMR spectrum of **4** in $(CD_3)_2SO$ solution (Figure 4 of the Supporting Information) revealed the presence of diagnostic crosspeaks at F1/F2 = δ 10.15/8.22 and 9.62/8.08 ppm due to a moderate *J*-coupling interaction in the NHCHO fragment of the major, *Z* conformer and a very strong one in that of the minor, *E* conformer, respectively. For each of the 256 increment fids, four transients of 1024 data points were collected. The spectral window was 2488 Hz.

C. Biological Methods. Inhibition of ADA by FAIRs. A published procedure for the spectrophotometric enzyme assay of adenosine deaminase[22] was modified as follows. Stock solutions (1 mM) of Ado and FAIRs were prepared in potassium phosphate buffer (50 mM, pH 7.5). A stock solution (1.2819 units·mL^{-1}) of ADA was prepared by adding 4.6 μL (19.228 units) of the commercial enzyme suspension to 14995 μL of the buffer solution containing 0.1 mg/mL of bovine serum albumin. This enzyme stock solution was kept in an ice bath (0−5 °C) until immediately prior to performing each assay. Eppendorf micropipettes were used to assemble 3.000 mL assay mixtures in 1 cm path length UV cuvettes. These mixtures typically consisted of 50 μL of ADA stock solution (0.0214 units·mL^{-1}), 77, 138, 199, 261, or 306 μL of Ado stock solution (25, 45, 65, 85, and 100 μM), and 0, 150, 300, and 450 μL of inhibitor stock solution (0, 50, 100, and 150 μM), and the remainder buffer. The rate of Ado deamination was monitored at 25 ± 0.1 °C by observing the decrease in absorbance at 265 nm over a 300 s reaction window as referenced against a cuvette containing an inhibitor concentration identical to that in the sample cell. Assays were run in triplicate for each sample. The rates were converted to mM·s^{-1} by using 8.1 for the ratio of molar extinction coefficients between Ado and Ino. Inspection of a Lineweaver−Burk plot (Figure 6 of the Supporting Information) of the kinetic data (Table 2 of the Supporting Information) revealed that inhibition of ADA by **4** was competitive. Michaelis−Menten equation calculations produced a K_m for Ado of 27.5 ± 1.1 μM (lit.[22]

K_m 32.7 ± 1.9 μM), a V_{max} for Ado of 2.9 × 10^{-8} mol·s^{-1}·units^{-1} (lit.[22] V_{max} 293 ± 9 μmol/(min·mg)), and a K_i(app) for **4** of 42.9 ± 4 μM. A slope replot of the data revealed an abscissa intercept corresponding to a K_i(app) value of 40 μM.

Acknowledgment. This work was supported by grant GM44819 from the National Institutes of Health. Mr. Frederick G. Jacobs assisted with the purification protocol for **4**, and Mr. Shaun Stauffer and Ms. Christina Olivetti assisted with the ADA assays. 5-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (iP-AICARs) was a gift from Prof. Leroy B. Townsend, University of Michigan, Ann Arbor, MI. This article is dedicated to Dr. Nelson J. Leonard, California Institute of Technology, Pasadena, CA, on the occasion of his eightieth birthday.

Supporting Information Available: Selected multisolvent ^{1}H and ^{13}C NMR chemical shift data, ^{1}H/^{1}H ROESY 2D, ^{1}H/^{1}H COSY 2D, and dynamic ^{1}H NMR spectral plots, ADA inhibition kinetic data with Lineweaver−Burk plot, and crystallographic data, atomic coordinates, equivalent isotropic temperature factors, bond lengths, angles, torsion angles, anisotropic thermal displacements, ORTEP of the unit cell with hydrogen bonding for **4**, and ^{1}H NMR spectral plots for **10**, **11**, and **16**−**18** (26 pages). Ordering information is given on any current masthead page.

References

(1) Abbreviations: ADA, adenosine deaminase (EC 3.5.4.4); AIR carboxylase, phosphoribosylaminoimidazole carboxylase (EC 4.1.1.21); Ado, adenosine; Ino, inosine; AIR, 5-aminoimidazole ribotide; AIRs, 5-aminoimidazole riboside; CAIR, 4-carboxy-5-aminoimidazole ribotide; CAIRs, 4-carboxy-5-aminoimidazole riboside; N^5-CAIR, 5-(carboxyamino)imidazole ribotide; FAIRs, 5-(formylamino)imidazole riboside [5-(formylamino)-1-(β-D-ribofuranosyl)imidazole]; AICARs, 5-aminoimidazole-4-carboxamide riboside.

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JM970301S