8-Aza-immucillins as Transition-State Analogue Inhibitors of Purine Nucleoside Phosphorylase and Nucleoside Hydrolases

Gary B. Evans,*,† Richard H. Furneaux,† Graeme J. Gainsford,† John C. Hanson,‡ Gregory A. Kicska,§ Antony A. Sauve,§ Vern L. Schramm,§ and Peter C. Tyler†

Carbohydrate Chemistry, Industrial Research Limited, P.O. Box 31-310, Lower Hutt, New Zealand, Department of Biochemistry, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, New York 10461, and Brookhaven National Laboratory, Upton, New York 11973

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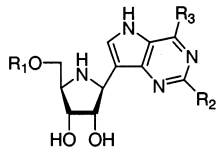
The 8-aza-immucillins (8-aza-9-deazapurines linked from C9 to C1 of 1,4-dideoxy-1,4-iminoribitol) have been designed as transition-state analogues of the reactions catalyzed by purine nucleoside phosphorylase and nucleoside hydrolases. Syntheses of the 8-aza-immucillin analogues of inosine and adenosine are described. They are powerful inhibitors of the target enzymes with equilibrium dissociation constants as low as 42 pM.

Introduction

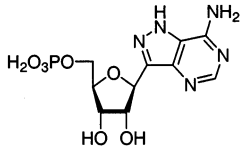
We have an ongoing interest in the design and synthesis of purine nucleoside phosphorylase (PNP) inhibitors because such compounds are expected to have therapeutic potential for the control of undesirable T-cell proliferation. The importance of PNP in T-cell proliferation has been demonstrated in patients with inherited PNP deficiency, where T-cell levels and their response to stimuli are dramatically reduced. However, these patients have relatively normal B-cell function.[1,2] PNP inhibition leads to an accumulation of deoxyguanosine (dG), which is metabolized to deoxyguanosine triphosphate (dGTP) in T-cells where it causes inhibition of ribonucleotide reductase and, consequently, DNA synthesis.[2,3] The human T-cell is unique in this selectivity because of high deoxycytidine kinase levels, which phosphorylate dG, and the relatively low nucleotidase activity, which allows dGTP to accumulate.[2]

Undesirable activation and proliferation of T-cells are associated with several human disease states including psoriasis, rheumatoid arthritis, T-cell leukemias and lymphomas, transplant tissue rejection, and other type IV autoimmune disorders. Consequently PNP inhibition has become a target for drug design.[4,5]

The transition-state structures of the enzyme-catalyzed phosphorolysis of inosine and the hydrolysis of inosine and adenosine have been solved by analysis of kinetic isotope effects.[6] The ribooxacarbenium character of the transition state can be reproduced in stable analogues by the combination of a 9-deazapurine and an iminoribitol. Using this information, we have designed and synthesized transition-state analogue inhibitors of PNP, protozoan nucleoside hydrolases, and phosphoribosyltransferases.[7−23] Immucillins **1** and **2** are exceedingly potent inhibitors of PNP, and **1** is in clinical trials for the control of T-cell leukemia. Additionally **1**−**3** are powerful inhibitors of nucleoside hydrolases, and the 5′-phosphates **4** and **5** are the most potent inhibitors known of several purine phosphoribosyltransferases.[9] In addition to powerful inhibition of their target enzymes, the immucillins have been useful in crystallography by providing complexes with closed and ordered catalytic sites. Properties of inhibition and catalytic site contacts support the proposal that these complexes are closely related to the structures at the transition states.[13,17,21]



1 Immucillin-H R_1=H, R_2=H, R_3=OH
2 Immucillin-G R_1=H, R_2=NH_2, R_3=OH
3 Immucillin-A R_1=H, R_2=H, R_3=NH_2
4 Immucillin-HP R_1=PO_3H_2, R_2=H, R_3=OH
5 Immucillin-GP R_1=PO_3H_2, R_2=NH_2, R_3=OH



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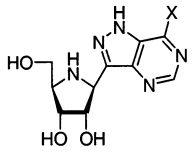

7 X = OH
8 X = NH_2

Early studies on transition-state analogues of AMP *N*-ribosylhydrolase revealed that formycin 5′-phosphate **6** is a powerful inhibitor.[24,25] As part of ongoing studies into the structure−activity relationship of the immucillins, we were interested in how 8-aza-immucillins **7** and **8** might perform as inhibitors of PNP and nucleoside hydrolases. The extra nitrogen would be expected to modify the pK_a of the 7-NH residue, an important site for substrate−enzyme interaction in both PNP and nucleoside hydrolases.

We report here the first synthesis of 8-aza-immucillins **7** and **8** and their in vitro activity against bovine and human PNP and two nucleoside hydrolases.

Results and Discussion

Synthesis. The immucillins have all been synthesized by way of imine **9** (Scheme 1). Thus, immucillin-H

* To whom correspondence should be addressed. Phone: 64-4-569-0000. Fax +64-4-569-0055. E-mail g.evans@irl.cri.nz.
† Industrial Research Limited.
‡ Brookhaven National Laboratory.
§ Albert Einstein College of Medicine of Yeshiva University.

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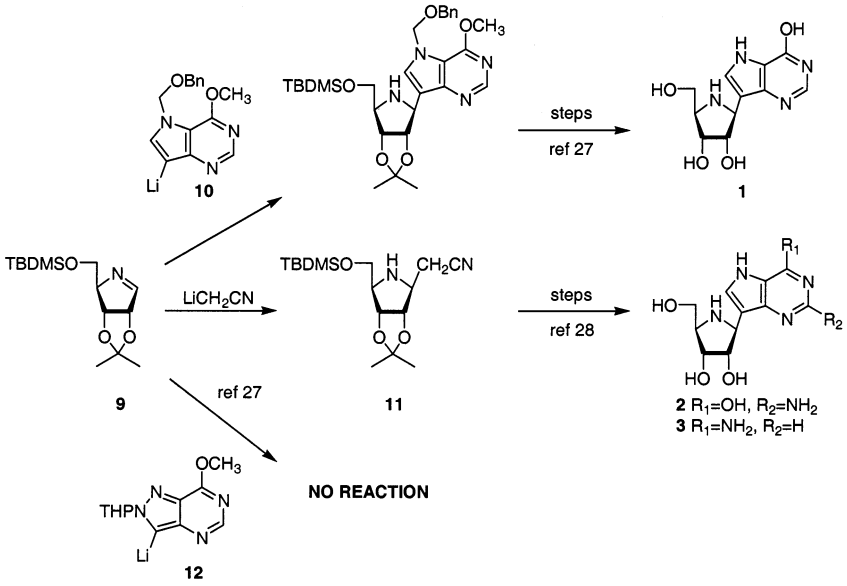
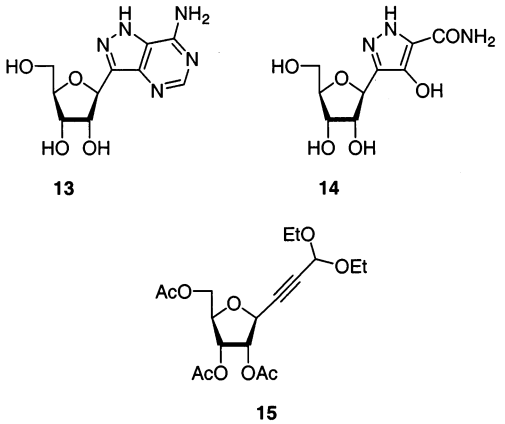
Scheme 1



(**1**) may be readily prepared by a direct condensation of the lithiated deazapurine derivative **10** with imine **9**.[26] The 9-deazaguanosine and -adenosine analogues **2** and **3** are better prepared via the acetonitrile adduct **11**[27] as lithiated 9-deazaadenine and 9-deazaguanine derivatives undergo decomposition either in concert with or before addition to the imine **9**. Similarly, attempts to prepare **7** by addition of the lithiated pyrazolopyrimidine derivative **12** to the imine **9** were unsuccessful.[26]

By analogy with reported work on the synthesis of formycin (**13**) and pyrazofurin (**14**) from the acetylene *C*-glycoside (**15**),[28−31] we envisaged that the addition of an appropriate lithiated alkyne to imine **9** would afford material that could be transformed into the desired products.



Addition of lithiated 3,3-diethoxy-1-propyne to the imine **9** (Scheme 2) at 10−15 °C in diethyl ether afforded the aza-*C*-glycoside **16**. Reaction temperatures above 15 °C led to the rapid decomposition of the lithiated alkyne, but below 10 °C little or no addition to the imine was seen. With careful control of the temperature, the reaction could be carried out on a multigram scale and in acceptable yield. Treatment of **16** with 2,2,2-trichloroethyl chloroformate afforded **17**. The Troc protecting group was selected because it is stable at both extremes of pH necessary for subsequent manipulations and under nitration conditions.

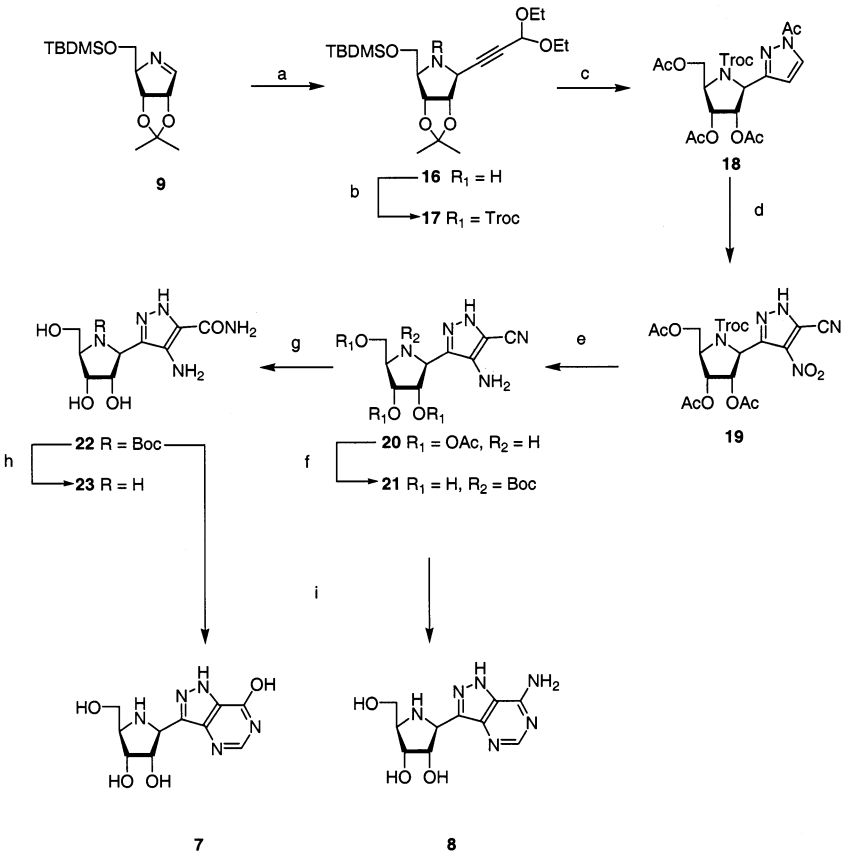
Deprotection of the diethyl acetal was achieved with a mixture of glacial acetic acid and 10% aqueous hydrochloric acid, and the resulting acetylenic aldehyde was treated in situ with hydrazine hydrate under reflux to afford a pyrazole. The crude product was acetylated to give the peracetylated pyrazole derivative **18** in good overall yield.

Nitration of **18** was achieved using ammonium nitrate and trifluoroacetic anhydride (TFAA) in trifluoroacetic acid (TFA)[31] to afford a 2,4-dinitropyrazole, which was treated without purification with potassium cyanide in a mixture of ethanol and ethyl acetate to afford the 2-cyano-3-nitropyrazole **19** in excellent yield. Reduction of the nitro group of **19** to give the desired aminopyrazole **20** proved to be problematic because standard hydrogenation conditions either were ineffective or produced side products. This may in part be due to the reported instability of the Troc group under hydrogenolysis conditions.[32] However, zinc in acetic acid efficiently reduced the nitro group, although these conditions also removed the Troc group to give **20**. The acetate protecting groups were removed at this stage because some of the reagents required for later steps resulted in their partial loss. Deacetylation under Zemplen conditions followed by reprotection of the iminoribitol nitrogen as the *tert*-butyl carbamate gave the key intermediate **21**.

Hydrolysis of nitrile **21** was effected by treatment with hydrogen peroxide and potassium carbonate in DMSO, affording amide **22** in good yield. Deprotection of **22** with dilute HCl afforded the seco-immucillin analogue **23** as its hydrochloride salt. Treatment of **21** with formamidine acetate in refluxing ethanol followed by dilute HCl afforded the 8-aza-immucillin-A homologue **8** as its hydrochloride salt. Similar treatment of **22** afforded the 8-aza-immucillin-H homologue **7** as its hydrochloride salt.

X-ray Single-Crystal Analysis of 8-Aza-immucillin-H (7). An X-ray diffraction analysis of 8-aza-immucillin-H (**7**) (Figure 1) confirmed its structure, providing proof that the product derived by addition of

Scheme 2[a]



[a] Reagents: (a) 3,3-diethoxy-1-lithiopropyne, diethyl ether, 10−15° C, 46%; (b) 2,2,2-trichloroethyl chloroformate, Hunigs base, CH_2Cl_2, 0 °C → room temp, 88%; (c) glacial acetic acid, 10% HCl, room temp, then N_2H_4, reflux, then Ac_2O, Py, room temp, 65%; (d) TFAA, TFA, NH_4NO_3, 0 °C → room temp, then KCN, EtOAc, EtOH, room temp, 93%; (e) Zn, HOAc, room temp, 68%; (f) NaOMe, MeOH, room temp, 84%, then Boc_2O, MeOH, room temp, 71%; (g) H_2O_2, K_2CO_3, DMSO, room temp, 61%; (h) H_3O^+, room temp, 100%; (i) formamidine acetate, ethanol, reflux, then H_3O^+, room temp.

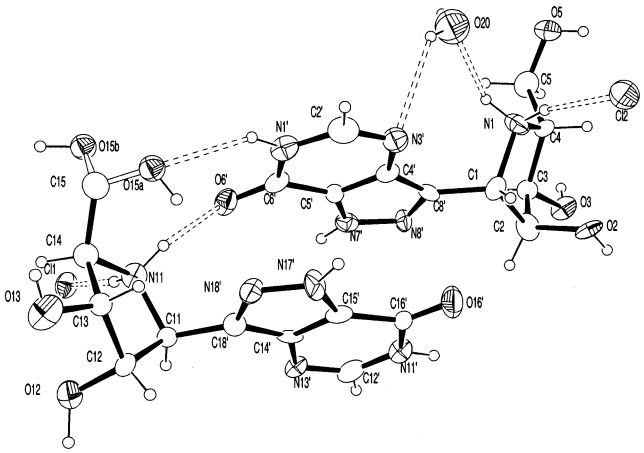


Figure 1. X-ray single-crystal analysis of 8-aza-immucillin-H (**7**).

the lithiated alkyne to imine **9** possessed the required β-stereochemistry.

Inhibition of Purine Nucleoside Phosphorylases by 8-Aza-immucillins. Inhibition of bovine and human PNP by 8-aza-immucillin-H **7** and **23** were tested and compared to immucillin-H **1** and immucillin-G **2** (Table 1). For the human enzyme, **1**, **2**, **7**, and **23** all inhibit within a factor of 4, with K_i* values of 29−104 pM. The same series for the bovine enzyme reveals K_i* values of 23−60 pM. The pyrazolo group, as opposed to a pyrolo group, weakens the equilibrium binding to both the human and bovine enzymes by approximately a factor of 2. The similar inhibition of these two enzymes arises from common transition-state interactions, since the human and bovine enzymes share 87% identity in the amino acid sequence, and in the X-ray crystal structures, contacts to substrate and product analogues in human and bovine enzymes are identical within experimental error.[33] The seco-analogue **23** has surprisingly robust activity considering that the rigid structure of the purine ring has been lost. The major inhibitory forces between PNP and the immucillins therefore arise from bonds readily achieved even with rotation of the carboxamide group. The N-3 in **1**, **2**, and **7** can interact only as an H-bond acceptor, while in **23** it is restricted to H-bond donation. The similar affinity indicates that enzymatic interaction with N-3 plays a minor role in K_i*.

Table 1. Inhibition Constants for the Interaction of Immucillins with Human and Bovine PNPs

	human PNP		bovine	
inhibitor	K_i* (pM)	k_6 (s^{-1})	K_i* (pM)	k_6 (s^{-1})
1	72 ± 26 [a]	(1.5 ± 0.6) × 10^{-3} [a]	23 ± 5 [a]	(4 ± 2) × 10^{-5} [a]
2	29 ± 8 [a]	(1.5 ± 0.6) × 10^{-3} [a]	30 ± 6 [a]	(1.9 ± 0.2) × 10^{-4} [a]
7	104 ± 31	(1.3 ± 0.1) × 10^{-2}	42 ± 6	(2.1 ± 0.1) × 10^{-2}
23	96 ± 16	(1.3 ± 0.1) × 10^{-3}	60 ± 50	(1.9 ± 0.1) × 10^{-2}

[a] Values taken from ref 12.. The rate constant k_6 describes the conformational change from the tightly bound EI* complex to the less tightly bound EI complex for the equilibrium $E + I \rightleftharpoons EI \underset{k_6}{\rightleftharpoons} EI^*$. K_i* is the dissociation constant for $E + I \rightleftharpoons EI^*$.

Table 2. Inhibition Constants for the Interaction of Immucillins with IU and IAG Nucleoside Hydrolases

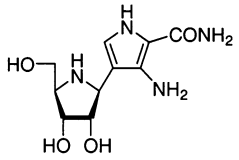
	IU nucleoside hydrolase K_i* or K_i (μM)	IAG nucleoside hydrolase K_i* or K_i (μM)
1	0.042 [a]	0.024 [a]
3	0.0072 [a]	0.0009 [a]
24	0.003 [a]	0.023 [a]
7[b]	1.6 ± 0.8	13.9 ± 0.8
8[b]	0.15 ± 0.1	14.0 ± 1.0
23[b]	4.8 ± 0.7	3.7 ± 0.5

[a] Values taken from ref 15. Compounds **1, 3**, and **24** cause tight binding inhibition characterized by K_i*. [b] Compounds **7**, **8**, and **23** are competitive inhibitors where K_i represents dissociation constants for E + I ⇌ EI.

Immucillin-G **2** remains the most powerful inhibitor known for human PNP. Changes in pK_a values at the pyrazolo nitrogens and the hydrogen bonding capacity of the exocyclic oxygen are likely to be responsible for the small decrease in affinity of **7** relative to immucillin-G **2** and immucillin-H **1**.

8-Aza-immucillin-A **8** was, as expected, inactive against both PNP enzymes. Adenosine is not a substrate for these PNP enzymes but is a weak substrate for bacterial PNPs.

Inhibition of Nucleoside Hydrolases by 8-Aza-immucillins. Compounds **7**, **8**, and **23** were tested as inhibitors of the nonspecific inosine−uridine nucleoside hydrolase (IU−NH) from *Crithidia fasciculata* and of the purine-specific inosine−adenosine−guanosine nucleoside hydrolase (IAG-NH) from *Trypanosoma brucei brucei* in comparison to immucillin-H **1**, immucillin-A **3**, and **24** (Table 2). For the enzyme from *C. fasciculata*,



24

7, **8**, and **23** are poorer inhibitors by 1−3 orders of magnitude, with inhibition constants from 0.15 to 4.8 μM. The same series for the *T. brucei brucei* enzyme reveals K_i values of 14.0, 13.9, and 3.7 μM, all significantly weaker than for immucillin-H **1**, immucillin-A **3**, and **24**.

Conclusions

8-Aza-immucillins are powerful inhibitors for mammalian PNPs with K_i* values near 100 pM. The PNP isozyme specificity is similar for 8-aza-immucillins and the parent immucillins. 8-Aza-immucillin-A **8**, as expected, was inactive against human and bovine PNP and had weaker equilibrium binding to the nucleoside hydrolase enzymes than **3** by 3−4 orders of magnitude. Perturbation of N-7 interactions with target enzymes by introduction of the pyrazolo group has little effect on PNPs but has profound effects on the nucleoside hydrolases. These features will be useful in the design of transition-state analogues for specific *N*-ribosyltransferases.

Experimental Section

1. General. Each new compound migrated as a single discrete component on thin-layer chromatography using aluminum-backed silica gel sheets (Merck or Reidel de Haen). NMR spectra were recorded on a Bruker AC-300 instrument at 300 MHz (^{1}H) or 75 MHz (^{13}C). Normally, spectra were measured in $CDCl_3$ with Me_4Si as the internal reference; when D_2O was the solvent, acetone (^{1}H, δ 2.20; ^{13}C, δ 33.2) was used as the internal reference. High-resolution accurate mass determinations were performed by Hort Research Ltd., Palmerston North, New Zealand, on a VG70-250S double-focusing magnetic sector mass spectrometer under chemical ionization conditions using isobutane or ammonia as the ionizing gas or under high-resolution fast atom bombardment conditions in a glycerol or nitrobenzyl alcohol matrix. Melting points were determined on a Reichert hot stage microscope and are uncorrected. Column chromatography was performed on silica gel (230−400 mesh, Merck). Chromatography solvents were distilled prior to use.

2. Chemistry. (1*S*)-5-*O*-*tert*-Butyldimethylsilyl-1,4-dideoxy-1-(3,3-diethoxyprop-1-ynyl)-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (16). A solution of (1*S*)-5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol[26] (6.0 g, 21.0 mmol) in petroleum ether (100 mL) was stirred with *N*-chlorosuccinimide (3.6 g, 27.0 mmol) for 1 h. The solids were removed by filtration, and the solvent was removed in vacuo. The resulting residue was dissolved in dry THF (100 mL), and the solution was cooled to −78 °C. Lithium tetramethylpiperidide (75 mL, 0.4 M in THF) was added dropwise, and on completion the reaction was diluted with petroleum ether (200 mL) and washed with water (100 mL) and brine (100 mL). The organic layer was dried, concentrated, and chromatographed to afford imine **9** (4.70 g) as a colorless oil. The imine **9** was dissolved in diethyl ether (75 mL), and the resulting solution was then added via cannula to one of the lithiated 3,3-diethoxypropynes [prepared by the dropwise addition of *n*-butyllithium (24.0 mL, 57.6 mmol) to a solution of 3,3-diethoxypropyne (2.08 mL, 40 mmol) in dry diethyl ether (150 mL) at −78 °C, followed by stirring for 15 min] at −78 °C. The reaction mixture was stirred for 10 min at −78 °C and then allowed to warm to 15 °C and held at this temperature for 1 h. After this time, the reaction was quenched by the addition of water (5 mL) and the mixture was partitioned into water and ethyl acetate. The organic phase was dried, concentrated, and chromatographed to afford alkyne (**16**) (4.00 g, 9.7 mmol, 46%) as an oil. IR ν_{max}: 3448 (NH), 2243 (C≡C), 1462, 1372, 1254 cm^{-1}. ^{1}H NMR: δ 5.19 [1H, s, $(CH_3O)_2CH$], 4.60 (1H, dd, $J_{2,3}$ = 6.3, $J_{1,2}$ = 3.2 Hz, H-2), 4.50 (1H, dd, $J_{2,3}$ = 6.3, $J_{3,4}$ = 2.8 Hz, H-3), 3.87 (1H, d, $J_{1,2}$ = 3.2 Hz, H-1), 3.59 (6H, m, OCH_2, H-5), 3.18 (1H, dd, $J_{4,5}$ = 5.9, $J_{3,4}$ = 2.8 Hz, H-4), 1.42, 1.25 [2 × 3H, s, $C(CH_3)_2$], 1.14 (2 × 3H, t, J = 4.8 Hz, OCH_2CH_3), 0.83 [9H, s, $C(CH_3)_3$], 0.08 (6H, s, $SiCH_3$). ^{13}C NMR: δ 113.1 [$OC(CH_3)_2$], 91.3 [$CH(OEt)_2$], 86.7 (C-2), 85.0 (C═C), 82.5 (C-3), 79.8 (C═C), 66.7 (C-4), 63.2 (C-5), 60.8 (OCH_2CH_3), 55.5 (C-1), 27.0, 25.0 [$C(CH_3)_2$], 25.9 [$C(CH_3)_3$], 18.3 [$C(CH_3)_3$], 15.1 (OCH_2CH_3), −5.5, −5.4 ($SiCH_3$). HRMS (M^+) calcd for $C_{21}H_{39}NO_5Si$: 414.2676. Found: 414.2684.

(1*S*)-5-*O*-*tert*-Butyldimethylsilyl-1,4-dideoxy-1-(3,3-diethoxyprop-1-ynyl)-1,4-imino-2,3-*O*-isopropylidene-*N*-(2,2,2-trichloroethoxycarbonyl)-D-ribitol (17). 2,2,2-Trichloroethyl chloroformate (3.0 mL, 22 mmol) was added dropwise to a solution of amine **16** (6.0 g, 14.5 mmol) and Hunig's base (7.5 mL, 43 mmol) in dichloromethane (50 mL) at 0 °C. The resulting solution was allowed to warm to room temperature, stirred for an additional 30 min, and then partitioned between chloroform and 10% HCl. The organic layer was further washed with water and brine, dried, concentrated, and chromatographed to afford **17** (7.5 g, 12.7 mmol, 88%) as a syrup. ^{1}H NMR (C_6D_6): δ 5.22 [1H, brs, $(CH_3O)_2CH$], 4.96 (2H, m, H-2, H-3), 4.55 (2H, s, CH_2CCl_3), 4.46 (1H, brs, H-1), 3.80 (6H, m, OCH_2, H-5), 3.46 (1H, m, H-4), 1.35, 1.12 [2 × 3H, brs, $C(CH_3)_2$], 1.14 (2 × 3H, brs, OCH_2CH_3), 0.97 [9H, s, $C(CH_3)_3$], 0.13, 0.11 (2 × 3H, s, $SiCH_3$). ^{13}C NMR (C_6D_6): δ 152.6 (NCO), 112.4 [$OC(CH_3)_2$], 96.1 (CCl_3), 91.8 [$CH(OEt)_2$], 86.0, 84.9 (C-2), 83.0, 82.1 (C-3), 82.9, 81.6 (C═C), 75.1 (OCH_2CCl_3), 67.3, 66.9 (C-4), 62.6, 62.2 (C-5), 61.0 (OCH_2CH_3), 56.6, 56.4 (C-1), 27.1, 25.0 [$C(CH_3)_2$], 26.1 [$C(CH_3)_3$], 18.5 [$C(CH_3)_3$], 15.3

(OCH_2CH_3), −5.5, −5.4 ($SiCH_3$). HRMS (M^+) calcd for $C_{24}H_{40}Cl_3NO_7Si$: 587.1640. Found: 587.1541.

(1*S*)-2,3,5-Tri-*O*-acetyl-1-(1-acetyl-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-*N*-(2,2,2-trichloroethoxycarbonyl)-D-ribitol (18). Acetal (**17**) (7.5 g, 12.8 mmol) was dissolved in glacial acetic acid (125 mL) and 10% HCl (30 mL), and the mixture was stirred at room temperature for 1 h. To this, a solution of hydrazine hydrate (7.5 mL, 155 mmol) in glacial acetic acid (60 mL) was added dropwise over 10 min. The resulting solution was heated at reflux for 16 h and concentrated in vacuo to afford a dark-brown oil. The crude product was redissolved in pyridine (60 mL), acetic anhydride (30 mL) was added, and the resulting solution was stirred for 16 h at room temperature. Solvent was removed in vacuo, and the crude residue was redissolved in ethyl acetate (500 mL), washed with 10% HCl (100 mL), water (100 mL), and brine (100 mL), dried, concentrated, and chromatographed to afford **18** (4.5 g, 8.3 mmol, 65%) as a syrup. ^{1}H NMR (C_6D_6): δ 7.95 (1H, brd, J 10.5 Hz, H-5′), 6.36 (1H, brd, J 10.5 Hz, H-4′), 5.95 (2H, m, H-2, H-3), 5.33 (1H, d, J = 26.3 Hz, H-1), 4.60 (2H, m, H-5), 4.40 (2H, m, CH_2CCl_3), 1.68 (3H, s, $NCOCH_3$), 1.67 (9H, s, $OCOCH_3$). ^{13}C NMR (C_6D_6): δ 169.6, 169.3, 169.1, 168.8 ($COCH_3$), 154.4, 154.1 (C-3′), 153.6 (NCO), 129.2 (C-5′), 109.2, 108.6 (C-4′), 95.7 (CCl_3), 75.2 (OCH_2CCl_3), 75.2, 74.1 (C-2), 73.0, 72.3 (C-3), 62.3, 62.0 (C-5), 61.0, 60.5 (C-1), 60.3, 60.1 (C-4),21.1, 20.1 (4 × $COCH_3$). HRMS (MH^+) calcd for $C_{19}H_{23}Cl_3N_3O_9$: 542.0500. Found: 542.0513.

(1*S*)-2,3,5-Tri-*O*-acetyl-1-(5-cyano-4-nitro-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-*N*-(2,2,2-trichloroethoxycarbonyl)-D-ribitol (19). Trifluoroacetic anhydride (12.0 mL, 85 mmol) was added dropwise to a stirred solution of **18** (4.5 g, 8.3 mmol) and ammonium nitrate (6.8 g, 85 mmol) in trifluoroacetic acid (100 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for an additional 3 h. The reaction was then diluted with chloroform, washed with water until the pH of the aqueous layer was neutral, dried, and concentrated in vacuo. The crude product thus isolated was committed to the next step without further purification. A solution of the 2,4-dinitro compound (5.0 g) in ethanol (45 mL) and ethyl acetate (45 mL) was added dropwise over 5 min to a stirred solution of potassium cyanide (6.6 g, 101 mmol) in ethanol (120 mL) and water (30 mL). Following an additional 5 min at room temperature, the reaction mixture was neutralized with acetic acid and diluted with ethyl acetate (500 mL), washed with water (100 mL) and brine (100 mL), dried, concentrated in vacuo, and chromatographed to afford **19** (4.4 g, 7.7 mmol, 93%) as a syrup. IR ν_{max}: 3345 (NH), 2229.8 (CN), 1741 (CO), 1376, 1235, 1048 cm^{-1}. ^{1}H NMR (C_6D_6): δ 6.04 (1H, d, J = 6.0 Hz, H-1), 5.69 (1H, brs, H-2), 5.42 (1H, brs, H-3), 4.54−3.75 (5H, m, H-4, H-5, CH_2CCl_3), 1.83, 1.79, 1.71 (3H, s, $COCH_3$). ^{13}C NMR (C_6D_6): δ 172.8, 169.8, 169.6 ($COCH_3$), 153.4 (NCO), 143.1, 134.5, 123.7 (C-3′, C-4′, C-5′), 111.0 (CN), 94.9 (CCl_3), 75.4 (OCH_2CCl_3), 75.4 (C-2), 71.8 (C-3), 63.6 (C-5), 62.9 (C-4), 57.1 (C-1), 20.6, 20.1, 19.9 ($COCH_3$). HRMS (MH^+) calcd for $C_{18}H_{18}Cl_3N_5O_{10}$: 570.0198. Found: 570.0181.

(1*S*)-2,3,5-Tri-*O*-acetyl-1-(4-amino-5-cyano-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol (20). Zinc dust (1.5 g) was suspended in glacial acetic acid (10 mL) with stirring, and a solution of pyrazole **19** (570 mg, 1 mmol) in glacial acetic acid (5 mL) was then added dropwise over a 5 min period. After a further 10 min, the zinc salts were removed by filtration and washed with ethyl acetate. The combined filtrate was concentrated in vacuo, redissolved in chloroform, washed with saturated sodium bicarbonate, water and brine, dried, concentrated in vacuo, and chromatographed to afford **20** (250 mg, 0.68 mmol, 68%) as a syrup. IR ν_{max}: 3345 (NH), 2229.8 (CN), 1741 (CO), 1376, 1235, 1048 cm^{-1}. ^{1}H NMR: δ 5.16 (1H, dd, J_{23} = 4.9, J_{12} = 3.5 Hz, H-2), 4.99 (1H, dd, $J_{3,4}$ = 7.2, $J_{3,4}$ = 4.9 Hz, H-3), 4.51 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 4.23 (2H, d, $J_{4,5}$ = 4.6 Hz, H-5), 3.77 (1H, dt, $J_{3,4}$ = 7.2, $J_{4,5}$ = 4.6 Hz, H-4), 2.16, 2.10, 2.10 ($COCH_3$). ^{13}C NMR: δ 171.5, 171.2, 170.3 ($COCH_3$), 133.0, 133.0, 129.3 (C-3′, C-4′, C-5′), 113.5 (CN), 75.9 (C-2), 72.1 (C-3), 65.1 (C-5), 59.0 (C-4), 57.5 (C-1), 21.2, 21.2, 21.0 (3 × $COCH_3$). HRMS (MH^+) calcd for $C_{15}H_{20}N_5O_6$: 366.1414. Found: 366.1424.

(1*S*)-1-(4-Amino-5-cyano-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol. A catalytic amount of sodium methoxide was added to a stirred solution of **20** (110 mg, 0.3 mmol) in methanol (3 mL) at room temperature. After 30 min, the reaction was complete and the product was preabsorbed onto silica and purified by chromatography to afford (1*S*)-1-(4-amino-5-cyano-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol (60 mg, 0.25 mmol, 84%). ^{1}H NMR (MeOH-d_4): δ 4.30 (1H, d, J = 7.2 Hz, H-1), 4.05−3.94 (2H, m, H-2, H-3), 3.65 (2H, dt, J = 11.1, J = 5.1 Hz, H-5), 3.27 (1H, q, J = 4.5 Hz, H-4). ^{13}C NMR (MeOH-d_4): δ 134.7, 134.7, 134.1 (C-3′, C-4′, C-5′), 114.9 (CN), 77.9 (C-2), 74.0 (C-3), 66.6 (C-4), 64.9 (C-5), 59.5 (C-1). HRMS (MH^+) calcd for $C_9H_{13}N_5O_3$: 239.1018. Found: 239.1028.

(1*S*)-1-(4-Amino-5-cyano-1*H*-pyrazol-3-yl)-*N*-(*tert*-butoxycarbonyl)-1,4-dideoxy-1,4-imino-D-ribitol (21). Di-*tert*-butyl dicarbonate (100 mg, 0.46 mmol) was added to a stirred solution of (1*S*)-1-(4-amino-5-cyano-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol (60 mg, 0.25 mmol) in methanol (5 mL) at room temperature. After 1 h, the reaction was complete and the reaction mixture was concentrated in vacuo and chromatographed to afford **21** (60 mg, 0.18 mmol, 71%), which was used in the subsequent step without characterization.

(1*S*)-1-(3-Amino-2-carboxamido-1*H*-pyrazol-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol (23). Hydrogen peroxide (0.3 mL) was added dropwise to a solution of **21** (60 mg, 0.18 mmol) and potassium carbonate (60 mg) in DMSO (1.0 mL), and the mixture was stirred for 1 h. The mixture was diluted with water (50 mL) and lyophilized, redissolved in water, preabsorbed onto silica, concentrated, and chromatographed to afford a product (42 mg) that was dissolved in 2 M HCl (5 mL). The solution was allowed to stand at room temperature for 5 min, and then the solvent was removed to afford the pyrazole *C*-nucleoside **23** as its hydrochloride salt (28 mg, 0.11 mmol, 61%). ^{1}H NMR (D_2O): δ 4.93 (1H, d, J = 7.7 Hz, H-1), 4.66 (1H, m, H-2), 4.43 (1H, t, J = 4.0 Hz, H-3), 3.92 (3H, m, H-4, H-5). ^{13}C NMR (D_2O): δ 163.3 ($CONH_2$), 134.4, 133.2, 118.5 (pyrazole), 73.9 (C-2), 71.0 (C-3), 66.1 (C-4), 58.9 (C-5), 55.6 (C-1). HRMS (MH^+) calcd for $C_9H_{16}N_5O_4$: 258.1202. Found: 258.1203.

(1*S*)-1-(7-Amino-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol (8·HCl). A solution of **21** (0.16 g, 0.47 mmol) in ethanol (5 mL) was stirred with formamidine acetate (0.06 g, 0.58 mmol) under reflux for 30 min. The solution was preabsorbed onto silica, and the solvent was removed in vacuo and chromatographed to afford a product (90 mg) that was dissolved in 2 M HCl (5 mL). The mixture was allowed to stand at room temperature for 5 min. The solvent was removed in vacuo to afford (1*S*)-1-(7-amino-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol·HCl (**8**·HCl) (76 mg, 0.25 mmol, 53%) as a hygroscopic white solid (recrystallized from MeOH/acetonitrile) that melted at 216 °C. $[\alpha]_D$ −9.2° (*c* 0.5, MeOH). IR ν_{max}: 3448 (NH), 1676, 1612, 1405, 1129 cm^{-1}. ^{1}H NMR (D_2O): δ 8.42 (1H, s, H-5′), 5.20 (1H, d, J = 6.5 Hz, H-1), 4.80 (1H, t, J = 4.6 Hz, H-2), 4.50 (1H, t, J = 4.6 Hz, H-3), 4.01−3.89 (3H, m, H-4, H-5a,-5b). ^{13}C NMR: δ 151.0 (C), 145.3 (C-5′), 134.9 (C), 132.9 (C), 123.4 (C), 73.9 (C-2), 70.9 (C-3), 65.6 (C-4), 58.8 (C-5), 57.3 (C-1). HRMS (MH^+) calcd for $C_{10}H_{15}N_6O_3$: 267.1206. Found: 267.1208. Anal. ($C_{10}H_{14}N_6O_3$·2HCl) C, H, N, Cl.

(1*S*)-1-(7-Hydroxy-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol (7·HCl). A solution of **22** (0.05 g, 0.19 mmol) in ethanol (5 mL) was stirred with formamidine acetate (0.05 g, 0.48 mmol) under reflux for 1 h. The solution was preabsorbed onto silica, and the solvent was removed in vacuo and chromatographed to afford a product (40 mg) that was dissolved in 2 M HCl (5 mL). The mixture was allowed to stand at room temperature for 5 min. The solvent was removed in vacuo to afford (1*S*)-1-(7-hydroxy-1*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)-1,4-dideoxy-1,4-imino-D-ribitol·HCl (**7**·HCl) (31 mg, 0.10 mmol, 54%) as a white solid (recrystallized from MeOH) that decomposed at 265 °C. $[\alpha]_D$ −25.0° (*c* 0.1, MeOH). IR ν_{max}: 3380 (NH), 1718, 1672, 1593,

1131, 1031 cm^{-1}. ^1H NMR (D_2O): δ 8.09 (1H, s, H-5′), 5.11 (1H, d, J = 6.2 Hz, H-1), 4.78 (1H, t, J = 5.0 Hz, H-2), 4.46 (1H, t, J = 5.0 Hz, H-3), 3.96−3.88 (3H, m, H-4, H-5a,5b). ^{13}C NMR: δ 154.8 (C), 144.9 (C-5′), 137.3 (C), 137.1 (C), 128.6 (C), 73.7 (C-2), 71.0 (C-3), 65.2 (C-4), 58.8 (C-5), 57.9 (C-1). HRMS (MH^+) calcd for $C_{10}H_{14}N_5O_4$: 268.1046. Found: 268.1055. Anal. ($C_{10}H_{14}N_5O_4$·HCl) C, H, N, Cl.

3. Biology. Enzymatic assays used the absorbance change as inosine is converted to hypoxanthine or the coupled assay of xanthine oxidase to convert hypoxanthine to uric acid.[12] Slow onset inhibition was analyzed by curve fitting.[34] Human and bovine PNP were purchased from Sigma, and nucleoside hydrolases were prepared from expression of the appropriate DNA in *E. coli* overexpression vectors.[15]

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Supporting Information Available: X-ray analysis of **7** and a table of hydrogen bond contact parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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