remaining portion was pipetted into a new tube, and the process was repeated, with samples being taken at the intervals shown in Table III.

The samples so obtained were analyzed by HPLC using 10-μL injections, with peak areas or peak heights being used to obtain the ratios.

Determination of the Solubilities of Compounds 5a–d. The indicated amount (Table II) for each drug, **5a–d**, was mixed with 1 mL of phosphate-buffered saline (9.195 g of sodium dihydrogen phosphate hydrate, 5.20 g of sodium chloride in 1 L water, adjusted to pH 7.3) and agitated at 37 °C (Eppendorf Model 5432 mixer and Model 5320 thermostat). At the specified times, the samples were centrifuged at 14000 ± 500 rpm for 5 min, and 25-μL samples were taken, diluted with 0.975 mL of HPLC mobile phase, and assayed by HPLC against known standard concentrations of the drugs. The results are tabulated in Table II.

Acknowledgment. The authors thank R. P. Crews (University of Alabama) for his assistance in the chemical aspects of this work, C. N. Richmond (University of Alabama) for the NMR spectra, and K. Sugibayashi (University of Utah) for his help with the solubility and partition coefficient determinations. E. Smal is acknowledged for the drawings. This work was supported, in part, by grants (AI-17456 and AI-14987) from the National Institutes of Health. W.J.L. was supported by a J. K. Lilly Memorial Fellowship from the American Foundation for Pharmaceutical Education.

Registry No. 1, 5536-17-4; 2, 82870-42-6; 3, 82845-91-8; 4a, 87970-03-4; 4b, 87970-04-5; 4c, 87970-05-6; 4d, 87984-85-8; 5a, 65174-95-0; 5b, 65174-99-4; 5c, 87970-06-7; 5d, 87970-07-8; *tert*-butylchlorodimethylsilane, 18162-48-6; 9-[5-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine, 87970-08-9.

Supplementary Material Available: Complete ^{1}H NMR data for compounds **4a–d** (Table V) and **5a–d** (Table VI) (2 pages). Ordering information is given on any current masthead page.

Adenosine Deaminase Inhibitors. Synthesis of Deaza Analogues of *erythro*-9-(2-Hydroxy-3-nonyl)adenine

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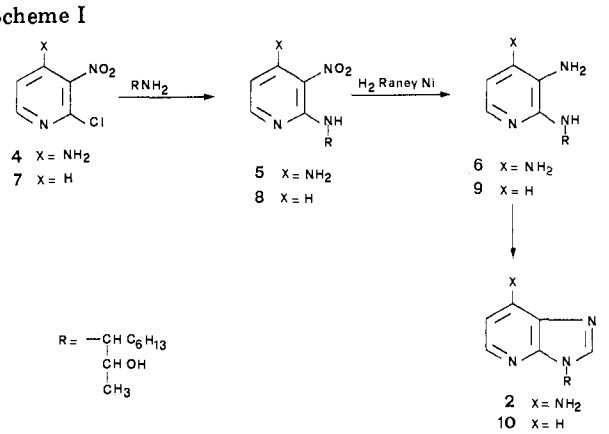
Received May 26, 1983

Structural analogues of *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA), in which the adenine moiety of the molecule was modified, were prepared in order to investigate the structural requirement of EHNA as an inhibitor of adenosine deaminase (ADA). Thus, 1- and 3-deaza-EHNA and their 6-deamino analogues were synthesized and evaluated as inhibitors of ADA from calf intestine. Inhibition studies indicated that isosteric substitution of pyrimidine nitrogens by carbons could be tolerated at the enzymatic binding site. In fact, 3-deaza-EHNA was found to have an inhibitory activity comparable to EHNA itself, and 1-deaza-EHNA, though less potent, is a good inhibitor. The 6-amino group gives an important contribution to the enzymatic binding if the N^1 nitrogen is also present, conferring on the compound the characteristic of a semitight inhibitor.

The ubiquitous enzyme adenosine demainase (ADA) catalyzes the hydrolytic N^6-deamination of adenine nucleoside analogues of chemotherapeutic interest to inactive or considerably less active hypoxanthine derivatives.[1] It was demonstrated that ADA inhibitors are able to enhance the cytotoxic activity of a variety of adenosine analgoues, such adenosine arabinoside (*ara*-A), 8-azaadenosine, and formycin.[2] Among these ADA inhibitors, pentostatin[3,4] and coformycin,[5] two naturally occurring nucleoside antibiotics, are extremely potent (K_i = 10^{-11} to 10^{-12} M).[6,7] Interest has been generated in tight-binding inhibitors of ADA because, in addition to potentiating the effect on nucleoside-type antitumor or antiviral agents, they may induce cellular deficiency of ADA and allow one to study of the effect of genetic deficiency of the enzyme and to clarify its function in severe combined immunodeficiency syndrome.[8] Among the synthetic compounds, the most active inhibitor is the *erythro*-9-(2-hydroxy-3-nonyl)-adenine (EHNA, 1),[9] which has been classified as a semitight-binding inhibitor with a K_i = 1.6 × 10^{-9} M.[7] As a result of the much faster reactivation of inhibited ADA observed in the case of inhibition by EHNA than that by pentostatin and coformycin, EHNA is advocated as a possible inhibitor of choice for use in viral chemotherapy.[10]

The tight binding of EHNA and of a previous series of 9-hydroxyalkylpurines might occur in an auxiliary site near that which normally binds the ribose moiety of adenosine. In the course of their research on nonnucleosidic adenine

Scheme I



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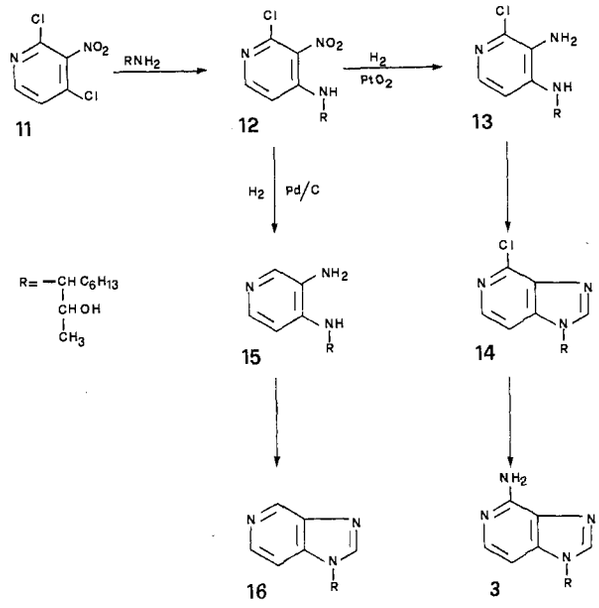
Scheme II



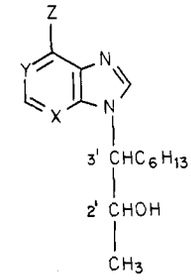
derivatives that led to discovery of EHNA, Schaeffer and Schwender have extensively studied and specified several factors that are important in complexation of the 9-substituent to the enzyme.[9] Until now, little attention has been paid to the heterocyclic moiety of EHNA, except for the work of Woo and Baker, which has provided some insight into its geometric orientation and the contribution of the 6-amino group to binding.[11]

To examine which structural parameters in the heterocyclic moiety are critical for inhibitory potency, we initiated a program to prepare EHNA analogues in which purine nitrogens are substituted in turn by carbons. In this paper, we report the synthesis and the ADA inhibitory activity of two deaza analogues of EHNA: the *erythro*-9-(2-hydroxy-3-nonyl)-1-deazaadenine (1-deaza-EHNA, **2**) and the *erythro*-9-(2-hydroxy-3-nonyl)-3-deazaadenine (3-deaza-EHNA, **3**). In addition, we have prepared and tested the corresponding deazapurine derivatives *erythro*-9-(2-hydroxy-3-nonyl)-1-deazapurine (**10**) and *erythro*-9-(2-hydroxy-3-nonyl)-3-deazapurine (**16**).

Chemistry. The synthesis of 1-deaza-EHNA (**2**) was accomplished by the method outlined in the Scheme I. 4-Amino-2-chloro-3-nitropyridine (**4**)[12] was condensed with *erythro*-3-amino-2-nonanol[9] to give compound **5**, which, after catalytic reduction to *erythro*-2-[(2-hydroxy-3-nonyl)amino]-3,4-diaminopyridine (**6**), was cyclized to 1-deaza-EHNA (**2**) by means of formamidine acetate. The structure of compound **2** was assigned on the basis of the ^{1}H NMR data in deuteriochloroform, which showed the presence of a NH_2 singlet at δ 5.33 exchangeable with D_2O and the absence of signals corresponding to the NH doublet in **5** (δ 8.88) and **6** (δ 4.74).

The synthesis of *erythro*-9-(2-hydroxy-3-nonyl)-1-deazapurine (**10**) was performed by the same method as above (Scheme I), starting from 2-chloro-3-nitropyridine (**7**). The substituted diaminopyridine **9** was cyclized with triethyl orthoformate, in the presence of a catalytic amount of ethanesulfonic acid, to the desired compound **10**.

Table I. Inhibition Constants of Calf Intestinal Adenosine Deaminase (ADA)



no.	X	Y	Z	K_i, M	K_m, M
1[a]	N	N	NH_2	7.0×10^{-9}	
2[a]	N	CH	NH_2	1.6×10^{-7}	
3[a]	CH	N	NH_2	1.0×10^{-8}	
10[a]	N	CH	H	5.5×10^{-7}	
16[a]	CH	N	H	1.2×10^{-6}	
17[a]	N	N	H	8.4×10^{-7}	
18 (1-deazaadenosine)				6.6×10^{-7} [b]	
19 (3-deazaadenosine)				3.6×10^{-4} [b]	
20 (purine riboside)				1.5×10^{-5} [b]	
21 (1-deazapurine riboside)				4.2×10^{-5} [b]	
22 (3-deazapurine riboside)				not active[b]	
adenosine					3.3×10^{-5}

[a] Mixture of two isomers: 2'*S*,3'*R* and 2'*R*,3'*S*. [b] Reference 17.

The syntheses of 3-deaza-EHNA (**3**) and *erythro*-9-(2-hydroxy-3-nonyl)-3-deazapurine (**16**) were carried out by the sequence shown in Scheme II. 2,4-Dichloro-3-nitropyridine (**11**)[13] was condensed with *erythro*-3-amino-2-nonanol to obtain the monoamination product **12**. The structure proof of **12** was obtained by catalytic hydrogenolysis of the chloro group and simultaneous reduction of the nitro group to give 3-amino-4-(2-hydroxy-3-nonyl)aminopyridine (**15**), an intermediate in the synthesis of **16**. The ^{1}H NMR spectrum of **15** showed the presence of a doublet at δ 6.45 (H-5) and a doublet and a singlet at δ 7.63 (H-6 and H-2); this excludes the possibility that the product might be the other isomer **9**. In fact, the latter ^{1}H NMR data included a double doublet at δ 6.49 (H-5) and two doublets at δ 7.57 (H-4 and H-6, respectively). Catalytic reduction of **12** gave *erythro*-3-amino-4-[(2-hydroxy-3-nonyl)amino]-2-chloropyridine (**13**), which was cyclized by means of diethoxymethyl acetate to *erythro*-4-chloro-1-(2-hydroxy-3-nonyl)-1*H*-imidazo[4,5-*c*]pyridine (**14**). Treatment of **14** with hydrazine hydrate, followed directly by reduction of the resulting 4-hydrazino compound with Raney nickel, afforded the desired 3-deaza-EHNA (**3**).

Ring closure of **15** with triethyl orthoformate in the presence of ethanesulfonic acid gave the 3-deazapurine derivative **16**.

Results and Discussion

The inhibitory action of EHNA primarily results from the strong binding of the *erythro*-nonyl moiety at an auxiliary region that normally might bind the ribose moiety of adenosine.[9,14,15] However, Woo and Baker have dem-

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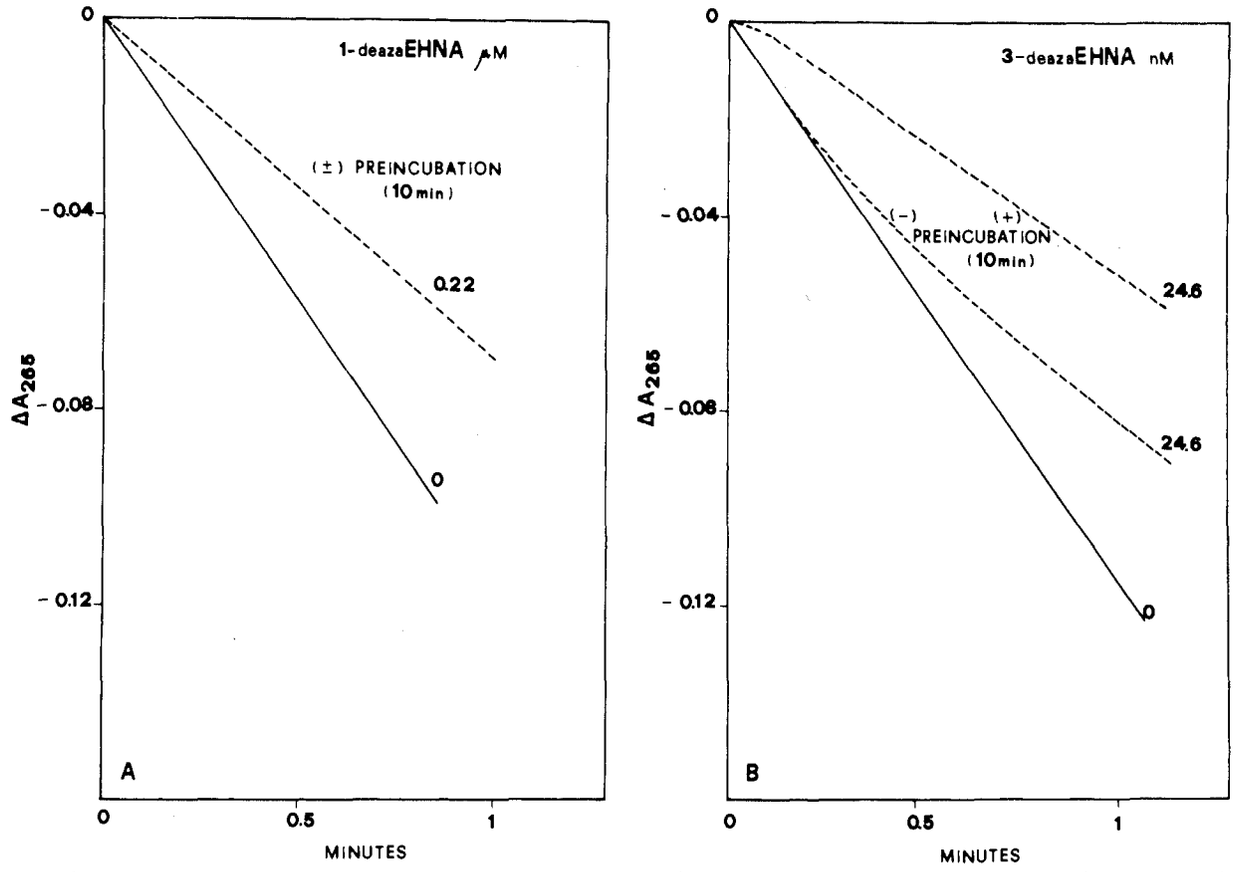

Figure 1. Spectrophotometric tracing of the reaction of calf intestine adenosine deaminase in the presence of 1-deaza-EHNA (frame A) and 3-deaza-EHNA (frame B) with (+) and without (-) preincubation. The reaction mixture is a total volume of 2.0 mL contained phosphate buffer (0.05 M, pH 7.6), adenosine (3.12×10^{-5} M), calf intestine adenosine deaminase (0.033 U), and inhibitors (concentration as indicated in the figure).

onstrated that the auxiliary region, which they term the "EHNA-specific region", has, in fact, a low correspondence in binding geometry with the ribose binding region, orienting the purine moiety and the C-6 amino group of EHNA away from the enzyme site where deamination occurs.[11] On the basis of these considerations, we deduced that the heterocyclic moiety of EHNA might be partially modified without considerably compromising the inhibitory activity.

As shown by our results in Table I, the 3-deaza analogue of EHNA (**3**) is a potent inhibitor of ADA with a K_i value close to that of EHNA, and the 1-deaza analogue (**2**), though less potent, is a good inhibitor. Therefore, substitution of the pyrimidine ring of EHNA with a pyridine indeed causes no considerable loss of inhibitory potency. The results also indicate, in general, increased affinity for EHNA analogues when compared to the corresponding ribofuranosyl derivatives, with the noteworthy exception of the 1-deaza derivative.

Different times were required to reach the steady state between the enzyme and the inhibitors **2** and **3**, respectively. In fact, whereas in the case of compound **2** the steady state is attained almost instantaneously, compound **3** requires 2–3 min (Figure 1), behaving analogously to the semitight inhibitor EHNA.[7] The other compounds reported in Table I behave as readily reversible inhibitors, analogously to **2**.

With regard to the influence of the 6-amino group on the inhibitory activity of EHNA and its deaza analogues **2** and **3**, our data on the activity of the deaminate analogues **10**, **16**, and **17** suggest that this group gives an important contribution to the binding in the case of EHNA and 3-deaza-EHNA, whereas such a contribution is less important in the case of 1-deaza-EHNA.

The 6-amino group could exert its influence on ADA binding either through its unshared electron pair to the enzyme, possibly by a hydrogen bond from the enzyme to the amino group, or by means of the inductive effect that the group at the 6-position has on the purine nucleus.[16] Substitution of CH for the N^1 nitrogen, as in the 1-deazaribofuranosyl derivative **18**, might tighten the ADA binding of the adjacent 6-amino group, as compared with adenosine. Such tight binding may interfere with the binding of the *erythro*-nonyl hydrophobic chain by forcing it to an unfavorable environment. Alternatively, the 1-modified purine moiety itself could be forced, because of the binding by the hydrophobic chain, to find a new rearrangement in the purine binding site, thus preventing the 6-amino group from possible interaction with the protein. This last hypothesis is supported by the small change in affinity recorded for 1-deaza-EHNA (**2**) vs. its deaminated derivative **10**.

Conversely, it was observed that substitution of CH for the N^3 nitrogen weakens the interaction at the active site of ADA by 3-deazaadenosine (**19**), compared to the substrate adenosine.[17] Since N^3 is possibly involved in stabilization of ribofuranosyl binding with ADA, presumably through hydrogen bonding with the 5′-OH, rather than in recognition of the substrate,[18] its absence should not in-

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fluence the mode of binding of the EHNA derivative through the 6-amino group. The binding of the 6-amino group, combined with that of the *erythro*-nonyl side chain, may then explain the high affinity of 3-deaza-EHNA (**3**). In fact, the 6-amino group importantly contributes to the interaction in **3**, as demonstrated by the drop in affinity by two order of magnitudes as compared with **16**, where it is absent.

In conclusion it may be affirmed that the isosteric substitution of the pyrimidine nitrogens by carbons in the purine system of EHNA leads to compounds that are still powerful ADA inhibitors. In particular, the substitution of CH for the N^3 nitrogen involves no significant change in activity, whereas a similar substitution of the N^1 nitrogen gives a compound that is 23 times less active than EHNA. The 6-amino group of this type of inhibitor gives an important contribution to the binding to the enzyme, particularly when the N^1 nitrogen is also present.

Experimental Section

The melting points were determined with a Büchi apparatus and are uncorrected. The ^1H NMR spectra were obtained with a Varian EM-390 90-MHz spectrometer, using Me_4Si (tetramethylsilane) as internal standard. The UV spectra were recorded on a Cary 219 spectrophotometer. TLC were carried out on silica gel 60 F-254 (Merck) precoated TLC plates. For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are indicated by atomic symbols and are within ±0.4% of theoretical values.

***erythro*-4-Amino-2-[(2-hydroxy-3-nonyl)amino]-3-nitropyridine (5).** A mixture of 2.4 g (13.8 mmol) of 4-amino-2-chloro-3-nitropyridine (**4**),[12] 2.42 g (15.2 mmol) *erythro*-3-amino-2-nonanol,[9] 2.2 g of dry triethylamine, and44 mL of nitromethane was refluxed for 3 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in water and extracted several times with $CHCl_3$. The organic phase was dried (Na_2SO_4) and evaporated, and the residue was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH–NH_3 (85:13:2) gave 3 g of **5** as a yellow oil: yield 73.3%; ^1H NMR ($CDCl_3$) δ 3.98 (m, 1, CH-2), 4.32 (m, 1, CH-3), 5.47 (s, 1, OH), 6.02 (d, 1, $J_{5,6}$ = 6 Hz, H-5), 7.00–7.73 (br s, 2, NH_2-4), 7.61 (d, 1, $J_{5,6}$ = 6Hz, H-6), 8.88 (d, 1, J = 6 Hz, NH-2). Anal. ($C_{14}H_{24}N_4O_3$) C, H, N.

***erythro*-2-[(2-Hydroxy-3-nonyl)amino]-3,4-diaminopyridine (6).** To a solution of 3 g (10.1 mmol) of **5** in 150 mL of ethanol was added 3 g of Raney nickel (washed with water), and the mixture was shaken with hydrogen at 30 psi for 2 h. After the catalyst was removed, the filtrate was evaporated, and the residue was chromatographed on a silica gel column. Elution with MeOH–$CHCl_3$ (70:30) yielded 1.61 g (60%) of **6** as a pure solid: mp 109–111 °C; ^1H NMR ($CDCl_3$) δ 3.70–4.40 (m, 6, CH-2, CH-3, NH_2-3, and NH_2-4), 4.74 (d, 1, J = 5 Hz, NH-2), 6.04 (d, 1, $J_{5,6}$ = 6 Hz, H-5), 7.44 (d, 1, $J_{5,6}$ = 6 Hz, H-6). Anal. ($C_{14}H_{26}N_4O$) C, H, N.

***erythro*-9-(2-Hydroxy-3-nonyl)-1-deazaadenine (2).** An intimate mixture of 1.6 g (6.0 mmol) of **6** and 1.2 g of formamidine acetate was heated at 150 °C with stirring for 20 min. The resulting solid was chromatographed on a silica gel column eluting with EtOAc–MeOH–NH_3 (95:4:1) to give **2** as a chromatographically homogeneous oil: yield 1 g (60.3%); ^1H NMR ($CDCl_3$) δ 0.8 (t, 3, $J_{8,9}$ = 6 Hz, CH_3-9), 0.93–1.60 (m, 11, CH_3-1, CH_2-5, CH_2-6, CH_2-7, and CH_2-8), 2.03 (m, 2, CH_2-4), 4.27 (m, 2, CH-2 and CH-3), 5.33 (s, 3, NH_2-6 and OH), 6.41 (d, 1, $J_{1,2}$ = 6 Hz, H-1), 7.81 (s, 1, H-8), 7.94 (d, 1, $J_{1,2}$ = 6 Hz, H-2); UV (pH 7.6) λ_{max} 262 nm (ε 10 900). Anal. ($C_{15}H_{24}N_4O$) C, H, N.

The base was converted to the oxalate salt by mixing equimolar amounts of **2** and oxalic acid in *i*-PrOH. The solution was left overnight at 0 °C, and the solid that precipitated was recrystallized from *i*-PrOH containing 1% oxalic acid to give the analytically pure sample, mp 152–154 °C. Anal. ($C_{15}H_{24}N_4O \cdot C_2H_2O_4$) C, H, N.

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***erythro*-2-[(2-Hydroxy-3-nonyl)amino]-3-nitropyridine (8).** A mixture of 1 g (6.3 mmol) of 2-chloro-3-nitropyridine (**7**), 1.1 g (6.9 mmol) of *erythro*-3-amino-2-nonanol,[9] 1 g of dry triethylamine, and 20 mL of nitromethane was refluxed for 2 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in water and extracted several times with $CHCl_3$. The combined extracts were dried (Na_2SO_4) and evaporated in vacuo to an oily residue, which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (50:1) yielded 1.53 g (70%) of **8** as a yellow oil: ^1H NMR ($CDCl_3$) δ 3.93 (m, 2, CH-2 and OH), 4.43 (m, 1, CH-3), 6.67 (dd, 1, H-5), 8.19 (d, 1, J = 8 Hz, NH-2), 8.35 (d, 1, $J_{5,6}$ = 5 Hz, H-6), 8.45 (d, 1, $J_{4,5}$ = 8 Hz, H-4). Anal. ($C_{14}H_{23}N_3O_3$) C, H, N.

***erythro*-2-[(2-Hydroxy-3-nonyl)amino]-3-aminopyridine (9).** To a solution of 1.5 g (5.65 mmol) of **8** in 75 mL of ethanol was added 1 g of Raney nickel (washed with water), and the mixture was shaken with hydrogen at 30 psi for 2 h. After the catalyst was removed by filtration, the filtrate was evaporated to give **9** as a chromatographically homogeneous oil: yield 1.3 g (94.8%); ^1H NMR ($CDCl_3$) δ 3.70 (m, 1, CH-2), 4.00 (m, 1, CH-3), 4.00–4.90 (br s, 6, NH-2, NH_2-3 and OH), 6.49 (dd, 1, H-5), 6.88 (d, 1, $J_{4,5}$ = 7 Hz, H-4), 7.57 (d, 1, $J_{5,6}$ = 5 Hz, H-6). Anal. ($C_{14}H_{25}N_3O$) C, H, N.

***erythro*-9-(2-Hydroxy-3-nonyl)-1-deazapurine (10).** To a stirred solution of 0.7 g (2.78 mmol) of **9**, 15 mL of triethyl orthoformate, and 5 mL of $CHCl_3$ was added 0.1 g of ethanesulfonic acid. The mixture was stirred at room temperature for 45 min and then neutralized with saturated Na_2CO_3 solution and extracted several times with $CHCl_3$. The residue was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH-NH_3 (91:8:1) to give 0.51 g (70 %) of **10** as a chromatographically pure oil: ^1H NMR ($CDCl_3$) δ 0.8 (t, 3, $J_{8,9}$ = 6 Hz, CH_3-9), 0.95–1.53 (m, 11, CH_3-1, CH_2-5, CH_2-6, CH_2-7, and CH_2-8), 2.03 (m, 2, CH_2-4), 4.30 (m, 2, CH-2 and CH-3), 5.30–5.46 (br s, 1, OH), 7.22 (dd, 1, H-1), 8.04 (s, 1, H-8), 8.06 (d, 1, $J_{1,6}$ = 8 Hz, H-6), 8.32 (d, 1, $J_{1,2}$ = 5 Hz, H-2); UV (pH 7.6) λ_{max} 284 nm (ε 9700). Anal. ($C_{15}H_{23}N_3O$) C, H, N.

The base was converted to the oxalate salt, which melted after recrystallization from methanol–water at 124–125 °C. Anal. ($C_{15}H_{23}N_3O \cdot C_2H_2O_4$) C, H, N.

***erythro*-4-[(2-Hydroxy-3-nonyl)amino]-2-chloro-3-nitropyridine (12).** A solution of 4 g (20.7 mmol) of 2,4-dichloro-3-nitropyridine (**11**)[13] in 100 mL of nitromethane, 3.6 g (22.6 mmol) of *erythro*-3-amino-2-nonanol,[9] and 3.4 g of dry triethylamine was refluxed for 1 h. After concentration in vacuo, the residue was dissolved in water and extracted several times with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and evaporated to a residue, which was chromatographed on a silica gel column. Elution with EtOAc–*n*-C_6H_{14}–C_6H_6 (50:40:10) yielded 2.69 g (40%) of **12** as a yellow pure oil: ^1H NMR ($CDCl_3$) δ 2.76 (br s, 1, OH), 3.64 (m, 1, CH-2), 4.02 (m, 1, CH-3), 6.56 (d, 1, J = 8 Hz, NH-4), 6.82 (d, 1, J = 6 Hz, H-5), 7.92 (d, 1, $J_{5,6}$ = 6 Hz, H-6). Anal. ($C_{14}H_{22}ClN_3O_3$) C, H, N.

***erythro*-3-Amino-4-[(2-hydroxy-3-nonyl)amino]-2-chloropyridine (13).** A solution of 2.1 g (6.65 mmol) of **12** in 250 mL of ethanol was hydrogenated at 1 atm of pressure in the presence of PtO_2 for 1 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue was chromatographed on a silica gel column eluting with EtOAc–C_6H_6 (50:50) to eliminate same impurities and then with ethyl acetate in increasing concentration to give 1.5 g (79 %) of **13** as chromatographically pure material: ^1H NMR ($CDCl_3$) δ 3.40 (m, 1, CH-2), 3.70 (s, 3, NH_2-3 and OH), 3.95 (m, 1, CH-3), 4.54 (d, 1, J = 9 Hz, NH-4), 6.41 (d, 1, $J_{5,6}$ = 5 Hz, H-5), 7.60 (d, 1, $J_{5,6}$ = 5 Hz, H-6). Anal. ($C_{14}H_{24}ClN_3O$) C, H, N.

***erythro*-4-Chloro-1-(2-hydroxy-3-nonyl)-1*H*-imidazo[4,5-*c*]pyridine (14).** A mixture of 1.5 g (5.24 mmol) of **13** and 20 mL of diethoxymethyl acetate was allowed to stand at room temperature for 7 h before it was evaporated to dryness. The residue was dissolved in 2 N HCl and, 1 h later, neutralized with saturated Na_2CO_3 solution and then extracted several times with $CHCl_3$. The chloroform extracts were dried with anhydrous Na_2SO_4 and evaporated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with EtOAc gave 1.32 g (85 %) of **14** as a white pure solid: mp 90–92 °C; ^1H NMR ($CDCl_3$) δ 4.23 (m, 2, CH-2 and CH-3), 7.39 (d, 1, $J_{6,7}$ = 5 Hz, H-7).

8.12 (s and d partially overlapped, 2, H-2 and H-6). Anal. ($C_{15}H_{22}ClN_3O$) C, H, N.

***erythro*-9-(2-Hydroxy-3-nonyl)-3-deazaadenine (3).** A mixture of 0.9 g (3.04 mmol) of **14** and 18 mL of hydrazine hydrate was refluxed for 2 h in a nitrogen atmosphere. After the reaction mixture was evaporated to dryness, 40 mL of oxygen-free water and 3.7 g of Raney nickel catalyst were added to the residue, and the mixture was refluxed for 1 h. After the catalyst was removed by filtration and washed with boiling water, the aqueous layer was extracted several times with $CHCl_3$. The combined extracts were dried (Na_2SO_4) and evaporated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with EtOAc–MeOH (65:35) yielded 0.3 g (35 %) of **3** as a viscous pure solid: ^1H NMR ($CDCl_3$) δ 0.82 (t, 3, $J_{8,9}$ = 6 Hz, CH_3-9), 0.96–1.50 (m, 11, CH_3-1, CH_2-5, CH_2-6, CH_2-7, and CH_2-8), 2.06 (m, 2, CH_2-4), 4.13 (m, 2, CH-2 and CH-3), 4.66–5.73 (br s, 3, NH_2-4 and OH), 6.72 (d, 1, $J_{2,3}$ = 6 Hz, H-3), 7.72 (d, 1, $J_{2,3}$ = 6 Hz, H-2), 7.80 (s, 1, H-8); UV (pH 7.6) λ_{max} 262 nm (ϵ 10 500). Anal. ($C_{15}H_{24}N_4O$) C, H, N.

The product was converted to the oxalate salt, which was recrystallized from *i*-PrOH containing 1% oxalic acid: mp 213–215 °C. Anal. ($C_{15}H_{24}N_4O \cdot C_2H_2O_4$) C, H, N.

***erythro*-3-Amino-4-[(2-hydroxy-3-nonyl)amino]pyridine (15).** To a solution of 1 g (3.2 mmol) of **12** in 250 mL of methanol was added 3 g of 10% Pd/C, and the mixture was shaken with hydrogen at 30 psi for 3 h. The catalyst was removed by filtration, the filtrate was evaporated, and the residue was made basic with saturated Na_2CO_3 solution. The aqueous phase was extracted several times with EtOAc and then the combined extracts were dried (Na_2SO_4) and concentrated to dryness. The residue was chromatographed on a silica gel column eluting with EtOAc–MeOH–NH_3 (80:19:1) to give **15** as a chromatographically pure oil: yield 0.64 g (80%); ^1H NMR (Me_2SO-d_6) δ 3.32 (m, 1, CH-2), 3.70 (m, 1, CH-3), 4.54 (s, 3, NH_2-3 and OH), 5.05 (d, 1, J = 9 Hz, NH-4), 6.45 (d, 1, $J_{5,6}$ = 5 Hz, H-5), 7.63 (s, and d partially overlapped, 2, H-2 and H-6). Anal. ($C_{14}N_{25}N_3O$) C, H, N.

***erythro*-9-(2-Hydroxy-3-nonyl)-3-deazapurine (16).** A mixture of 0.6 g (2.38 mmol) of **15**, 13 mL of triethyl orthoformate, and 0.1 g of ethanesulfonic acid was refluxed for 2 h. The cooled solution was neutralized with saturated Na_2CO_3 solution and extracted several times with EtOAc. The organic layer was dried (Na_2SO_4) and evaporated in vacuo, and the residue was chromatographed on a silica gel column. Elution with EtOAc–MeOH (70:30) yielded 0.45 g (73%) of **16** as a pure oil: ^1H NMR ($CDCl_3$) δ 0.82 (t, 3, $J_{8,9}$ = 6 Hz, CH_3-9), 0.96–1.50 (m, 11, CH_3-1, CH_2-5, CH_2-6, CH_2-7, and CH_2-8), 2.10 (m, 2, CH_2-4), 3.36–3.80 (br s, 1, OH), 4.22 (m, 2, CH-2 and CH-3), 7.38 (d, 1, $J_{2,3}$ = 6 Hz, H-3), 8.09 (s, 1, H-8), 8.31 (d, 1, $J_{2,3}$ = 6 Hz, H-2), 8.96 (s, 1, H-6); UV (pH 7.6) λ_{max} 256 nm (ϵ 6200). Anal. ($C_{15}H_{23}N_3O$) C, H, N.

The base was converted to the oxalate salt, which was purified by recrystallization from *i*-PrOH containing 1% oxalic acid, mp 136–138 °C. Anal. ($C_{15}H_{23}N_3O \cdot C_2H_2O_4$) C, H, N.

Reagents and Enzyme Assay. Calf intestine adenosine deaminase was purchased from Boehringer Mannheim (specific activity 200 U/mg at 25 °C); adenosine was obtained from Sigma Chemical Co. The rate of deamination was determined at 25 °C, unless specified otherwise, by following the decrease in absorbance for the transformation of adenosine into inosine at 265 nm, with a Cary 219 spectrophotometer using cells with 1-cm path-length cuvettes. The reaction mixture contained 0.05 M potassium phosphate buffer, pH 7.6, from 0.62×10^{-5} to 3.12×10^{-5} M adenosine, 0.033 U of adenosine deaminase, and a fixed concentration of the inhibitor (from 200 to 300% of the eventually determined K_i value) to a final volume of 2 mL.

All the inhibitors were assayed to determine the time course of the enzymic reaction with and without preincubation in the presence of inhibitor at 30 °C in order to establish the class of inhibitor: readily reversible, semitight binding, or tight binding.[2]

When the enzyme was preincubated with a fixed concentration of inhibitor (+) for the time indicated, the reaction was started by the addition of substrate (adenosine); without preincubation (–), the reaction was started by the addition of ADA. The deamination rates for EHNA and 3-deaza-EHNA were determined at 30 °C after a 3-min preincubation period of enzyme with various inhibitor concentrations.

K_i was obtained from eq 1 by the method of Lineweaver and Burk.[19] Under our experimental conditions, the K_m obtained for adenosine was 33 μM.

$$K_p = K_m\left(1 + \frac{i}{K_i}\right) \quad (1)$$

Acknowledgment. This work was supported by the Italian Research Council (CNR). We thank A. Biondi for elemental analyses and F. Lupidi for NMR spectra.

Registry No. **2**, 87871-08-7; **2** oxalate, 87871-20-3; **3**, 87871-09-8; **3** oxalate, 87871-22-5; **4**, 2789-25-5; **5**, 87871-10-1; **6**, 87871-11-2; **7**, 5470-18-8; **8**, 87871-12-3; **9**, 87871-13-4; **10**, 87871-14-5; **10** oxalate, 87871-21-4; **11**, 5975-12-2; **12**, 87871-15-6; **13**, 87871-16-7; **14**, 87871-17-8; **15**, 87871-18-9; **16**, 87871-19-0; **16** oxalate, 87871-23-6; **17**, 81129-36-4; (±)-*erythro*-3-amino-2-nonanol, 87935-58-8; formamidine acetate, 3473-63-0; triethyl orthoformate, 122-51-0; diethoxymethyl acetate, 14036-06-7; adenosine deaminase, 9026-93-1.

(19) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.