Adenosine Deaminase Inhibitors. Synthesis and Biological Evaluation of 4-Amino-1-(2(S)-hydroxy-3(R)-nonyl)-1H-imidazo[4,5-c]pyridine (3-Deaza-(+)-EHNA) and Certain C1′ Derivatives†

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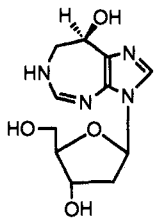
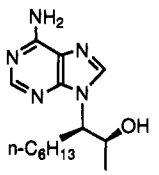
The synthesis of the title compound (15) and its 1′-fluoro (14) and 1′-hydroxy (12) derivatives is described. Key intermediate 10 was obtained by two routes through condensation of (2R,3R)-3-amino-1,2-O-isopropylidene-1,2-nonanediol (3) with either 2,4-dichloro- or 4-chloro-3-nitropyridine. When assayed as adenosine deaminase inhibitors, 15 was found to be almost twice as active as its racemate. While hydroxylation at the 1′-position resulted in an 80-fold decrease in activity, the 1′-fluoro derivative proved to have activity comparable to that of 3-deaza-(+)-EHNA.

Introduction

Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4) hydrolyzes adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively, and inactivates a number of antiviral and cancer chemotherapeutic adenosine analogs.[1,2] In ADA deficiency, catabolism of 2′-deoxyadenosine is blocked and a severe combined immunodeficiency disease (SCID) is caused by the accumulation of deoxy-ATP.[3,4] Consequently, inhibition of ADA can prevent inactivation of clinically useful adenosine analogs, and the administration of 2′-deoxycoformycin (1, dCF) mimics ADA deficiency for the treatment of lymphoproliferative disorders.[5,6] Another potential benefit of ADA inhibition is protection of injured tissues. Both cerebral and myocardial ischemia result in the release of adenosine, which appears to limit the extent of degeneration.[7,8] Two potent and selective ADA inhibitors, namely, dCF[9] and *erythro*-9-(2-hydroxy-3-nonyl)adenine (2, EHNA)[9-11] were found to protect against myocardial ischemia/reperfusion injury in animal models. These effects and other physiological roles of adenosine[12] have continued to stimulate interest in the development of ADA inhibitors.[13,14] Due to the immunosuppressive effects of irreversible ADA inhibition, most applications of adenosinergic therapy would call for a reversible inhibitor.



1, dCF



2, (+)-EHNA

An understanding of ADA interaction with a tight-binding transition-state inhibitor was recently provided by X-ray analysis.[15] However, an ADA complex with a semi-tight-binding inhibitor such as EHNA has not yet been described. The ADA X-ray structure revealed a hydrogen bond between Gly-84 and purine N3 that is postulated to help orient C6 and stabilize the transition state for hydrolytic deamination. Deletion of N3 in adenosine causes loss of substrate activity.[16] Because EHNA and related 9-substituted adenines are not substrates for ADA and because modifications at the 6-position of 9-alkyladenines are detrimental to binding although they enhance the affinity of adenosine,[17] the 6-position of EHNA is believed to orient away from the catalytic region.[18] The change in position appears to reduce the importance of N3 to binding, and indeed, 3-deaza-EHNA is also a semi-tight-binding inhibitor of ADA.[19] This and our earlier observation that N3 participates in intramolecular displacement of reactive substituents at C1′ [20] make the preparation of ADA inhibitors from 3-deaza-EHNA derivatives an attractive goal.

In our previous work, we investigated the structure–activity relationship (SAR) requirements of the 2′-hydroxyl and the adjacent binding sites of EHNA.[20] Our efforts to introduce a fluorine atom at C1′ were thwarted by intramolecular cyclization involving N3. We now report the synthesis and the ADA inhibitory activity of a series of optically active 3-deaza-EHNA analogs whose chirality is derived from L-ascorbic acid.[21]

Chemistry

The synthesis of the target compounds depended upon the preparation of key intermediate 10 (Scheme 1). Two routes were followed starting with the condensation of amine 3[21] with either 2,4-dichloro-[22] or 4-chloro-3-nitropyridine[23,24] (4 and 5). Compound 6 was obtained by a literature procedure.[22] Catalytic hydrogenation of 6 gave 3-aminopyridine 8 in good yield. This, upon treatment with triethyl orthoformate and a catalytic amount of concentrated HCl, produced 4-chloroimidazo[4,5-c]pyridine 10 in quantitative yield. Formation of the orthoformyl ester is precedented.[20]

In the second route, condensation of amine 3 with 5 afforded 7 in 75% yield. Treatment of 7 with stannous chloride in concentrated HCl resulted in simultaneous reduction of the nitro group, chlorination at C2, and cleavage of the isopropylidene ring to give 9 in 69% yield.

† Taken, in part, from Harriman, G. C. B. Ph.D. Dissertation, The University of Rhode Island, Kingston, RI, 1991.

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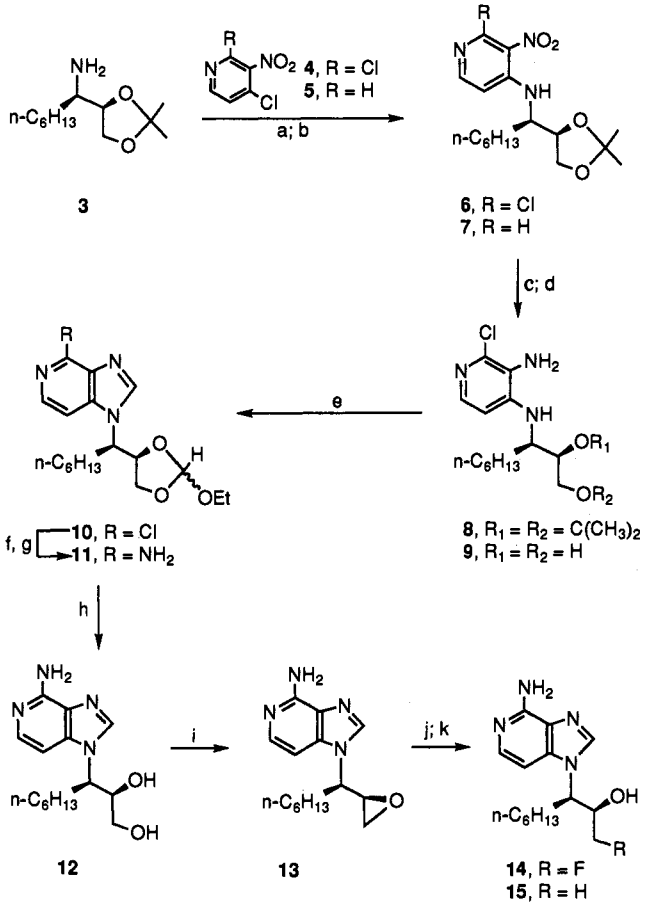
Scheme 1 [a]



[a] (a) **3** → **6**, Et_3N, $MeNO_2$, reflux, 44%; (b) **3** → **7**, *n*-Bu_3N, *n*-$C_5H_{11}OH$, 70 °C, 75%; (c) **6** → **8**, H_2/PtO_2, EtOH, 75%; (d) **7** → **9**, $SnCl_2$, HCl, 90 °C, 69%; (e) $HC(OEt)_3$, HCl, 95%; (f) 95% H_2NNH_2, reflux, 12 h; (g) Ra Ni, H_2O, 67%; (h) H^+ resin, EtOH, room temperature, 95%; (i) Ph_3P, DIAD, C_6H_6, Δ, 77%; (j) **13** → **14**, pyr(HF)$_n$, pyrn, room temperature → 35 °C, 45%; (k) **13** → **15**, $LiAlH_4$, THF, 0 °C, 37%.

Conversion of **9** to **10** proceeded under the identical reaction conditions described earlier. It is worth mentioning that the second route proved to be preferable to the first since compound **10** was obtained in higher overall yield (52% vs 33%).

Completion of the synthesis is also outlined in Scheme 1. Displacement of the 4-chloro group of **10** with hydrazine followed by Ra Ni-2800 reduction gave **11**. Water was found to be the solvent of choice in this reaction since the use of alcohols resulted in difficult filtration of the catalyst. Product **11** contained Ni which was removed by chelation with ethylenediamine. Hydrolysis of the ortho ester was accomplished with Amberlite IR-120 H^+ resin in 95% EtOH followed by the addition of saturated methanolic ammonia to give diol **12**. Epoxide **13** was obtained from diol **12** by the Mitsunobu reaction.[25] The ^1H NMR spectrum of **13** clearly showed a typical AMX pattern associated with monosubstituted epoxides.[26] Two target compounds, namely, fluorohydrin **14** and the 3-deaza analog of (+)-EHNA **15**, were then prepared from epoxide **13**. The former was obtained by treatment of **13** with pyridine poly(hydrogen fluoride)[27] and the latter by reduction with LAH. The introduction of fluorine in **14** at the 1′-position is evidenced by ^{19}F NMR which shows a triplet $J = 47$ Hz at δ −108.511. Analysis of the ^1H NMR spectrum showed the diastereotopic 1′-methylene protons as two doublets of a doublet of doublets centered at δ 4.23 and 4.38 with geminal and H–F coupling constants of 9.9 and 47 Hz, respectively. The 1′,2′-vicinal coupling constants were 4.0 and 5.1 Hz.

Table 1. Inhibition of Adenosine Deaminase

compound	K_i (nM)
12	497
14	4.7
15	6.2

Results and Discussion

The K_i values for inhibition of calf intestinal ADA by 3-deaza-(+)-EHNA and its C1′ derivatives are presented in Table 1. 3-Deaza-(+)-EHNA (**15**) inhibited ADA with a K_i of 6.2 nM, a value that is approximately one-half that reported for the racemic mixture (10 nM).[19] This result is consistent with our prior finding that the potent inhibitory activity of racemic EHNA resides in the (+)-form.[28] Also, as noted above, the 3-position of adenine plays a minimal role in EHNA binding, and the K_i value for 3-deaza-(+)-EHNA is only 3-fold higher than the 2 nM value for (+)-EHNA.[28]

It has been postulated that the 1′-methyl group of EHNA associates with a specific methyl-binding pocket.[29] Deletion or hydroxylation of this carbon atom causes respective 23- and 28-fold reductions in (+)-EHNA potency.[20] The C1′-hydroxy derivative **12** proved to be 80-fold less potent than 3-deaza-(+)-EHNA. Therefore, the 3-deaza modification may alter the orientation of the 9-substituent in such a way that a C1′-hydroxyl causes an additional 3-fold greater loss of affinity than is seen for the same modification with (+)-EHNA. On the other hand, fluorination at C1′ of 3-deaza-(+)-EHNA yielded a derivative, **14**, with a 4.7 nM inhibition constant. This compound, which has similar or slightly greater affinity than 3-deaza-(+)-EHNA, and *erythro*-9-(2(*S*),9-dihydroxy-3(*R*)-nonyl)adenine (9′-hydroxy-(+)-EHNA)[30] are the most potent (+)-EHNA analogs reported to date.

Experimental Section

Melting points were determined on a Buchi 535 melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a Varian EM-390 or a Bruker AM-300 spectrometer if so indicated. The chemical shifts are expressed in parts per million with respect to tetramethylsilane. Optical rotations were obtained with a Perkin-Elmer Model 141 digital readout polarimeter. Silica gel (Davison, grade H, 230–425 mesh), suitable for flash column chromatography, was purchased from Fisher Scientific. Thin-layer chromatography was run on precoated (0.2-mm) silica gel 60-F254 plates manufactured by EM Science, Inc., and short-wave UV light (254 nm) was used to detect the UV-absorbing spots. Visualization by charring with 50% sulfuric acid followed by heating was used to detect those compounds that were not UV active. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

2-Chloro-4-[(1,2(R)-dihydroxy-1,2-*O*-isopropylidene-3(*R*)-nonyl)amino]-3-nitropyridine (6). To a stirred solution of 2,4-dichloro-3-nitropyridine (**4**) (471 mg, 2.44 mmol) and triethylamine (0.34 mL, 2.44 mmol) in anhydrous nitromethane (15 mL) was added **3** (450 mg, 2.10 mmol). The reaction mixture was heated at reflux for 1 h. The solvent was removed, and pure **6** (345 mg, 44% yield) was obtained by silica gel flash chromatography eluting with ethyl acetate–hexanes (1:10): $[\alpha]^{26}_D$ −12.86° (*c* 2.10, EtOH); ^1H NMR ($CDCl_3$) δ 0.83 (*t*, J = 6 Hz, 3 H), 1.00-1.84 (m, 16 H), 3.53–3.82 (m, 2 H), 3.87–4.35 (m, 2 H), 6.16–6.40 (b d, J = 9 Hz, 1 H, D_2O exchangeable), 6.73 (d, J = 6.7 Hz, 1 H), 7.93 (d, J = 6.7 Hz, 1 H). Anal. ($C_{17}H_{26}ClN_3O_4$) C, H, N, Cl.

4-[(1,2(*R*)-Dihydroxy-1,2-*O*-isopropylidene-3(*R*)-nonyl)amino]-3-nitropyridine (7). To a stirred solution of 4-chloro-

3-nitropyridine (3) in tributylamine (3.34 mL, 14.0 mmol) was added 3 (3.11 g, 14.0 mmol) in anhydrous pentanol (20 mL). The reaction mixture was stirred at 70 °C for 10 h. Pure 7 was obtained as a viscous orange gum (3.54 g, 75% yield) by silica gel flash chromatography eluting with ethyl acetate–hexanes (1:1): $[\alpha]^{27}_D$ −27.23° (c 2.81, EtOH); ^{1}H NMR ($CDCl_3$) δ 0.82 (t, J = 4.5 Hz, 3 H), 1.08–1.88 (m, 16 H), 3.70–4.37 (m, 4 H), 6.90 (d, J = 6 Hz, 1 H), 8.11 (d, J = 9.0 Hz, 1 H, D_2O exchangeable), 8.20 (d, J = 6.0 Hz, 1 H), 9.12 (s, 1 H). Anal. ($C_{17}H_{27}N_3O_4$) C, H, N.

3-Amino-2-chloro-4-[(1,2(*R*)-dihydroxy-1,2(*R*)-*O*-isopropylidene-3(*R*)-nonyl)amino]pyridine (8). A solution of 6 (225 mg, 0.605 mmol) in absolute ethanol (50 mL) was hydrogenated over PtO_2 at atmospheric pressure for 1 h. The reaction mixture was filtered, and the solvent was removed. The residue was purified by silica gel flash chromatography eluting with ethyl acetate–hexanes (2:3) to give pure 8 as a gum (154 mg, 75% yield): $[\alpha]^{22}_D$ +8.46° (c 2.25, EtOH); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 6 Hz, 3 H), 1.00–1.78 (m, 14 H), 3.33–4.26 (m, 8 H, 2 D_2O exchangeable), 4.34–4.56 (b d, J = 9 Hz, 1 H, D_2O exchangeable), 6.50 (d, J = 6.7 Hz, 1 H), 7.66 (d, J = 6.7 Hz, 1 H). Anal. ($C_{17}H_{28}ClN_3O_2$) C, H, N, Cl.

3-Amino-2-chloro-4-[(1,2(*R*)-dihydroxy-3(*R*)-nonyl)amino]pyridine (9). $SnCl_2$ (2.84 g, 15 mmol) was carefully added in small portions (effervescence) to a solution of 7 (1.01 g, 2.99 mmol) in concentrated HCl (10 mL) at 90 °C and heating was continued for 0.5 h. After the addition of H_2O (50 mL) at 5 °C, the reaction mixture was evaporated to dryness. To the residue was added saturated methanolic ammonia until a precipitate formed. This was followed by an additional 10 mL of saturated methanolic ammonia. The solution was stored at 5 °C for 12 h and then filtered through Celite. The solvent was removed, and 10 mL of 10% HCl was added to the residue, which was passed through a Dowex 50W-H^+ resin column eluting with H_2O (300 mL) followed by saturated methanolic ammonia (300 mL). Basic fractions containing 9 (620 mg, 69% yield) were combined to give a tan solid upon removal of the solvent: mp 85.4–86.5 °C; $[\alpha]^{27}_D$ + 24.62° (c 1.95, EtOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, J = 6 Hz, 3 H), 1.10–1.72 (m, 10 H), 3.28–3.55 (m, 4 H), 4.55 (s, 1 H, D_2O exchangeable), 4.72 (s, 1 H, D_2O exchangeable), 4.78 (s, 2 H, D_2O exchangeable), 5.33 (d, J = 8.4 Hz, 1 H, D_2O exchangeable), 6.42 (d, J = 5.4 Hz, 1 H), 7.35 (d, J = 5.4 Hz, 1 H). Anal. ($C_{14}H_{24}ClN_3O_2$) C, H, N, Cl.

4-Chloro-1-[1,2(*R*)-dihydroxy-1,2-*O*-(ethoxymethylene)-3(*R*)-nonyl]-1*H*-imidazo[4,5-*c*]pyridine (10). To a stirred solution of 8 (1.19 g, 3.47 mmol) in triethyl orthoformate (TEOF, 25 mL) was added concentrated HCl (0.05 mL). The reaction mixture was stirred at room temperature for 24 h. TEOF was removed, and pure 8 (1.31 g, 95% yield) was obtained by silica gel flash chromatography eluting with ethyl acetate–hexanes (2:3): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, J = 6.5 Hz, 3 H), 0.91–1.38 (m, 11 H), 1.82–2.22 (m, 2 H), 3.38–4.73 (m, 6 H), 5.73 and 5.78 (s, 1 H), 7.32 (d, J = 6 Hz, 1 H), 8.01 and 8.04 (s, 1 H), 8.19 (d, J = 6 Hz, 1 H). Anal. ($C_{18}H_{26}ClN_3O_3$) C, H, N, Cl.

4-Amino-1-[1,2-(*R*)-dihydroxy-1,2-*O*-(ethoxymethylene)-3(*R*)-nonyl]-1*H*-imidazo[4,5-*c*]pyridine (11). A solution of 95% hydrazine (50 mL) and 10 (1.69 g, 4.59 mmol) was heated at reflux for 12 h. Excess hydrazine was removed, and the residue was treated with Ra Ni-2800 (2 g, wet weight) in H_2O (50 mL) and refluxed 1 h. The reaction mixture was filtered hot through a Celite pad and washed with hot water. The Celite pad was then washed with CH_2Cl_2. The residues from both filtrates were combined and heated at 50 °C in ethylenediamine (EDA, 5 mL) for 0.5 h. Excess EDA was removed, and pure 11 (1.10 g, 67% yield) was obtained as a gum by silica gel flash chromatography eluting with MeOH–EtOAc (1:50): ^{1}H NMR ($CDCl_3$) δ 0.78 (t, J = 6.5 Hz, 3 H), 0.92–1.39 (m, 11 H), 1.74–2.21 (m, 2 H), 3.33–4.65 (m, 6 H), 5.76 (s, 1 H), 5.98 (b s, 2 H, D_2O exchangeable), 6.65 (d, J = 6.0 Hz, 1 H), 7.74 (d, J = 6.0 Hz, 1 H), 7.82 (s, 1 H). Anal. ($C_{18}H_{28}N_4O_3$) C, H, N.

4-Amino-1-(1,2(*R*)-dihydroxy-3(*R*)-nonyl)-1*H*-imidazo[4,5-*c*]pyridine (12). A solution of 11 (589 mg, 1.69 mmol) in 95% EtOH was stirred with Amberlite IR-120 H^+ resin (1.0 g) at room temperature for 12 h. Methanolic ammonia (25 mL) was then added, and stirring was continued for 12 h. Filtration and removal of the solvent gave pure 12 as a gum (470 mg, 95% yield): $[\alpha]^{26}_D$ +18.10° (c 2.10, EtOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77 (t, J = 6 Hz, 3 H), 0.84–0.96 (m, 1 H), 0.98–1.22 (m, 7 H), 1.89–2.07 (m, 2 H), 3.14–3.30 (m, 2 H), 3.68–3.78 (m, 1 H), 4.27–4.34 (m, 1 H), 4.71 (b s, 1 H, D_2O exchangeable), 5.23 (b s, 1 H, D_2O exchangeable), 5.13 (b s, 2 H, D_2O exchangeable), 6.74 (d, J = 6.0 Hz, 1 H), 7.62 (d, J = 6.0 Hz, 1 H), 8.09 (s, 1 H). Anal. ($C_{15}H_{24}N_4O_2$) C, H, N.

4-Amino-1-(1,2(*R*)-epoxy-3(*R*)-nonyl)-1*H*-imidazo[4,5-*c*]pyridine (13). To a stirred solution of 12 (148 mg, 0.506 mmol) and triphenylphosphine (146 mg, 0.557 mmol) in anhydrous benzene (10 mL) was added diisopropyl azodicarboxylate (0.11 mL, 0.558 mmol). The reaction mixture was heated at reflux for 1 h. The solvent was removed and pure 13 (89 mg, 73% yield) and 12 (18 mg, 12%) were obtained by silica gel flash chromatography eluting with MeOH–EtOAc (1:33): mp 201 °C dec; $[\alpha]^{26}_D$ +35.35° (c 0.775, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 6.5 Hz, 3 H), 0.98–1.42 (m, 8 H), 1.62–2.20 (m, 2 H), 2.52–2.69 (dd, J = 4.5 and 3.0 Hz, 1 H), 2.80 (dd, J = 4.5 Hz, 1 H), 3.20–3.35 (m, 1 H), 3.93–4.25 (dd, J = 4.5 and 3.0 Hz, 1 H), 5.4 0 (b s, 2 H, D_2O exchangeable), 6.70 (d, J = 6 Hz, 1 H), 7.72 (s, 1 H), 7.79 (d, J = 6 Hz, 1 H). Anal. ($C_{15}H_{22}N_4O\cdot H_2O$) C, H, N.

4-Amino-1-(1-fluoro-2(*S*)-hydroxy-3(*R*)-nonyl)-1*H*-imidazo[4,5-*c*]pyridine (14). To a cold (−78 °C) stirred solution of pyridine poly(hydrogen fluoride) (1 mL) was added 13 (50 mg, 0.182 mmol) in anhydrous pyridine (1 mL). The reaction mixture was stirred at room temperature for 2 h followed by an additional 2 h at 35 °C. The reaction was terminated by the careful addition of a saturated aqueous sodium bicarbonate solution until effervescence ended. The reaction mixture was extracted with CH_2Cl_2 and purified by silica gel flash chromatography eluting with MeOH–EtOAc (3:97) to give 14 (24 mg, 45% yield) as a colorless foam: mp 49.5–51.7 °C; $[\alpha]^{26}_D$ +27.87° (c 1.05, EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (t, J = 6.7 Hz, 3 H), 1.07–1.28 (m, 8 H), 2.03–2.17 (m, 2 H), 4.12–4.16 (m, 1 H), 4.23 (ddd, $^2J_{HF}$ = 47.0, J_{gem} = 9.8, and J_{vic} = 5.18 Hz), 4.27–4.31 (m, 1H), 4.38 (ddd, $^2J_{HF}$ = 47.0, J_{gem} = 9.8, and J_{vic} = 4.0 Hz), 5.63 (b s, 3 H, D_2O exchangeable), 6.68 (d, J = 6 Hz, 1 H), 7.59 (d, J = 6 Hz, 1 H), 7.89 (s, 1 H). Anal. ($C_{15}H_{23}N_4OF$) C, H, N.

4-Amino-1-(2(*S*)-hydroxy-3(*R*)-nonyl)-1*H*-imidazo[4,5-*c*]pyridine (3-Deaza-(+)-EHNA) (15). To a cold (0 °C) stirred solution of lithium aluminum hydride (50 mg, 1.5 mmol) in anhydrous THF (1 mL) was added 13 (40 mg, 0.146 mmol) in anhydrous THF (5 mL). Water (0.5 mL) was carefully added, and the reaction mixture was filtered and evaporated. Pure 15 (15 mg, 37% yield) was obtained as a gum by flash chromatography eluting with MeOH–EtOAc (1:20): $[\alpha]^{25}_D$ +22.77° (c 1.00, EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.80 (t, J = 6.5 Hz, 3 H), 0.97–1.41 (m, 11 H), 1.58–2.20 (m, 2 H), 3.86–4.37 (m, 3 H, 1 D_2O exchangeable), 5.32 (b s, 2 H, D_2O exchangeable), 6.65 (d, J = 6 Hz, 1 H), 7.65 (d, J = 6 Hz, 1 H), 7.76 (s, 1 H). Anal. ($C_{15}H_{24}N_4O$) C, H, N.

Biological Evaluation. The analogs were tested as inhibitors of calf intestinal mucosa ADA. Deamination of adenosine to inosine at 30 °C was measured directly from the decrease in absorbance at 265 nM.[31,32] ADA (type VI), adenosine, and bovine albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO). The enzyme was diluted into a stabilizing buffer of 50 mM potassium phosphate, pH 7.0, containing 1 mg/mL bovine albumin. Varying concentrations of analogs were preincubated for 5 min with 0.1 mL of ADA in a total volume of 0.9 mL of phosphate buffer. This permitted the association reaction between the enzyme and a semi-tight-binding inhibitor to reach a steady state.[33] Reactions were started by the addition of 0.1 mL of substrate (final concentrations: 0.0083 unit/mL ADA, 38 μM adenosine, 50 mM phosphate). The K_i values were determined from nonlinear regression analysis of the velocity vs inhibitor concentration (I) curves using the Enzfitter computer program (Elsevier-BIOSOFT) for the equation $v = v_o - v_o I/[K_i(1 + S/K_m) + I]$, where v_o is the reaction rate in the absence of inhibitor. The K_m for adenosine, determined at 10–72 μM concentrations under identical conditions, was 25 μM.

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References

(1) Agarwal, R. P.; Cha, S.; Crabtree, G. W.; Parks, R. E., Jr. Coformycin and Deoxycoformycin: Tight-Binding Inhibitors of Adenosine Deaminase. In *Chemistry and Biology of Nucleosides and Nucleotides*; Harmon, R. E., Robins, R. K., and Townsend, L. B., Eds.; Academic Press: New York, 1978; pp 159–197.

(2) Haertle, T.; Carrera, C. J.; Wasson, D. B.; Sowers, L. C.; Richman, D. D.; Carson, D. A. Metabolism and Anti-human Immunodeficiency Virus-1 Activity of 2-Halo-2′,3′-dideoxyadenosine Derivatives. *J. Biol. Chem.* **1988**, *263*, 5870–5875.

(3) Giblett, E. R.; Anderson, J. E.; Cohen, F.; Pollara, B.; Meuwissen, H. J. Adenosine-deaminase Deficiency in Two Patients with Severely Impaired Cellular Immunity. *Lancet* **1972**, *2*, 1067–1069.

(4) Hirschhorn, R.; Roegner, V.; Rubinstein, A.; Papageorgiou, P. Plasma Deoxyadenosine, Adenosine, and Erythrocyte DeoxyATP are Elevated at Birth in an Adenosine Deaminase-deficient Child. *J. Clin. Invest.* **1980**, *65*, 768–771.

(5) Grever, M. R.; Siaw, M. F. E.; Jacob, W. F.; Niedhart, J. A.; Miser, J. S.; Coleman, M. S.; Hutton, J. J.; Balcerzak, S. P. The Biochemical and Clinical Consequences of 2′-Deoxycoformycin in Refractory Lymphoproliferative Malignancy. *Blood* **1981**, *57*, 406–417.

(6) Spiers, A. S. D.; Moore, D.; Cassileth, P. A.; Harrington, D. P.; Cummings, F. J.; Nieman, R. S.; Bennett, J. M.; O'Connell, M. J. Remissions in Hairy-cell Leukemia with Pentostatin (2′-Deoxycoformycin). *New Engl. J. Med.* **1987**, *316*, 825–830.

(7) Marangos, P. J. Adenosinergic Approaches to Stroke Therapeutics. *Med. Hypotheses* **1990**, *32*, 45–49.

(8) Engler, R. L. Adenosine. The Signal of Life? *Circulation* **1991**, *84*, 951–954.

(9) Dhasmana, J. P.; Digerness, S. B.; Geckle, J. M.; Ng, T. C.; Glickson, J. D.; Blackstone, E. H. Effect of Adenosine Deaminase Inhibitors on the Heart's Functional and Biochemical Recovery from Ischemia: A Study Utilizing the Isolated Rat Heart Adapted to ^{31}P Nuclear Magnetic Resonance. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 1040–1047.

(10) Zhu, O.; Chen, S.; Zou, C. Protective Effects of an Adenosine Deaminase Inhibitor on Ischemia - Reperfusion Injury in Isolated Perfused Rat Heart. *Am. J. Physiol.* **1990**, *259*, H835–H838.

(11) Dorheim, T. A.; Hoffman, A.; Van Wylen, D. G. L.; Mentzer, R. M., Jr. Enhanced Interstitial Fluid Adenosine Attenuates Myocardial Stunning. *Surgery* **1991**, *110*, 136–145.

(12) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine Receptors: Pharmacology, Structure-Activity Relationships, and Therapeutic Potential. *J. Med. Chem.* **1992**, *35*, 407–422.

(13) Shewach, D. S.; Krawczyk, S. H.; Acevedo, O. L.; Townsend, L. B. Inhibition of Adenosine Deaminase by Azapurine Ribonucleosides. *Biochem. Pharmacol.* **1992**, *44*, 1697–1700.

(14) Cristalli, G.; Eleuteri, A.; Vittori, S.; Volpini, R.; Camaioni, E.; Lupidi, G. Adenosine Deaminase Inhibitors: Structure–Activity Relationships in 1-Deazaadenosine and Erythro-9-(2-Hydroxy-3-Nonyl)Adenine Analogues. *Drug Dev. Res.* **1993**, *28*, 253–258.

(15) Wilson, D. K.; Rudolph, F. B.; Quiocho, F. A. Atomic Structure of Adenosine Deaminase Complexed with a Transition-State Analog: Understanding Catalysis and Immunodeficiency Mutations. *Science* **1991**, *252*, 1278–1284.

(16) Ikehara, M.; Fukui, T. Deamination of Adenosine Analogs with Calf Intestine Adenosine Deaminase. *Biochim. Biophys. Acta* **1974**, *338*, 512–519.

(17) Baker, B. R. *Design of Active-Site-Directed Irreversible Enzyme Inhibitors*; John Wiley & Sons: New York, 1967; pp 285–300.

(18) Woo, P. W. K.; Baker, D. C. Inhibitors of Adenosine Deaminase. Studies in Combining High-Affinity Enzyme-Binding Structural Units. *erythro*-1,6-Dihydro-6-(hydroxymethyl)-9-(2-hydroxy-3-nonyl)purine and *erythro*-9-(2-hydroxy-3-nonyl)purine. *J. Med. Chem.* **1982**, *25*, 603–605.

(19) Antonini, I.; Cristalli, G.; Franchetti, P.; Grifantini, M.; Martelli, S.; Lupidi, G.; Riva, F. Adenosine Deaminase Inhibitors: Synthesis of Deaza Analogues of *erythro*-9-(2-Hydroxy-3-nonyl)adenine. *J. Med. Chem.* **1984**, *27*, 274–278.

(20) Harriman, G. C. B.; Poirot, A. F.; Abushanab, E.; Midgett, R. M.; Stoeckler, J. D. Adenosine Deaminase Inhibitors. Synthesis and Biological Evaluation of C1′ and Nor-C1′ Derivatives of (+)-*erythro*-9-(2(*S*)-Hydroxy-3(*R*)-nonyl)adenine. *J. Med. Chem.* **1992**, *35*, 4180–4184.

(21) Abushanab, E.; Bessodes, M.; Antonakis, K. Practical Enantiospecific Synthesis of (+)-*erythro*-9-(2-Hydroxy-3-nonyl)adenine. *Tetrahedron Lett.* **1984**, *25*, 3841.

(22) Montgomery, J. A.; Hewson, K. J. 3-Deaza-6-methylthiopurine Ribonucleosides. *J. Med. Chem.* **1966**, *9*, 105–107.

(23) Wright, G. C. Rearrangement of Ethyl 2-(3-Amino-4-pyridinyl)-hydrazinecarboxylate Hydrochloride to 1-Amino-1*H*-imidazo[4,5-c]pyridin-2(3*H*)-one Hydrochloride. *J. Heterocycl. Chem.* **1976**, *13*, 601.

(24) Kruger, S.; Mann, F. G. Xanthones and Thioxanthones. Part VI. The Preparation and Properties of 9-Thia-3-aza-anthrone. *J. Chem. Soc.* **1955**, 2755.

(25) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1–28.

(26) Jackman, L. M.; Sternhell, S. *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: New York, 1969; pp 125–127.

(27) Olah, G. A.; Welch, J. T.; Vankar, Y. D.; Nojima, M.; Kerekes, I.; Olah, J. A. Synthetic Methods and Reactions. 63. Pyridinium Poly(hydrogen fluoride) (30% Pyridine-70% Hydrogen Fluoride): A Convenient Reagent for Organic Fluorination Reactions. *J. Org. Chem.* **1979**, *44*, 3872–3881.

(28) Bastian, G.; Bessodes, M.; Panzica, R. P.; Abushanab, E.; Chen, S.-F.; Stoeckler, J. D.; Parks, R. E., Jr. Adenosine Deaminase Inhibitors. Conversion of a Single Chiral Synthon into *erythro*- and *threo*-9-(2-Hydroxy-3-nonyl)adenines. *J. Med. Chem.* **1981**, *24*, 1383–1385.

(29) Schaeffer, H. J. Design and Evaluation of Enzyme Inhibitors, Especially of Adenosine Deaminase. *Topics Med. Chem.* **1972**, *3*, 1–24.

(30) Abushanab, E. In *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K., Baker, D. C., Eds.; Plenum Press: New York, 1993; p 170.

(31) Kalckar, H. M. Differential Spectrophotometry of Purine Compounds by Means of Specific Enzymes. III Studies of the Enzymes of Purine Metabolism. *J. Biol. Chem.* **1947**, *167*, 461–475.

(32) Agarwal, R. P.; Parks, R. E., Jr. Adenosine Deaminase from Human Erythrocytes. *Methods Enzymol.* **1978**, *51*, 502–507.

(33) Agarwal, R. P.; Spector, T.; Parks, R. E., Jr. Tight-Binding Inhibitors-IV. Inhibition of Adenosine Deaminase by Various Inhibitors. *Biochem. Pharmacol.* **1977**, *26*, 359–367.