# Adenosine Deaminase Inhibitors. Synthesis and Biological Evaluation of C1′ and Nor-C1′ Derivatives of (+)-*erythro*-9-(2(*S*)-Hydroxy-3(*R*)-nonyl)adenine†

Geraldine C. B. Harriman, Anne F. Poirot, and Elie Abushanab*

*Department of Medicinal Chemistry, University of Rhode Island, Kingston, Rhode Island 02881*

Rose Marie Midgett and Johanna D. Stoeckler

*Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912*

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The synthesis of various chiral derivatives of (+)-*erythro*-9-(2-hydroxy-3-nonyl)adenine, (+)-EHNA, from (2*S*,3*R*)-3-amino-1,2-*O*-isopropylidene-1,2-nonanediol by condensation with 5-amino-4,6-dichloropyrimidine is described. The compounds synthesized were C1′- and nor-C1′-(+)-EHNA derivatives. When tested with calf spleen ADA, C1′-OH- and nor-C1′-(+)-EHNA had comparable inhibitory activity that was 1 order of magnitude lower than that of (+)-EHNA. Potency was reduced further in nor-C1′ derivatives.

## Introduction

Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4) is a catabolic enzyme of the purine catabolic pathway that hydrolyzes adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. ADA has broad substrate specificity and deaminates a variety of adenosine analogs such as adenine arabinoside (*ara*-A), 8-azaadenosine, formycin,[1] and 2′,3′-dideoxyadenosine (ddA).[2] Deamination may inactivate adenosine analogs and limit their utility for antiviral and cancer chemotherapy. Additionally, ADA deficiency is associated with a severe combined immunodeficiency disease that is caused by lymphotoxic accumulations of 2′-deoxyadenosine and deoxy-ATP.[3,4] Thus, potent ADA inhibitors may be used in two ways: (1) to prevent the inactivation of chemotherapeutic adenosine analogs, thereby favoring their direct conversion to biologically active nucleotides, and (2) to treat lymphoproliferative disorders[5-8] by mimicking ADA deficiency.

The most potent inhibitors of ADA are coformycin (CF), 2′-deoxycoformycin (dCF, Pentostatin), and *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA). The former two naturally occurring nucleosides are base-modified transition-state analogs classified as tight-binding inhibitors with $K_i$ values of $1 \times 10^{-11}$ M (CF) and $2.5 \times 10^{-12}$ M (dCF).[1,9] EHNA, on the other hand, is a synthetic semitight binding inhibitor ($K_i = 4 \times 10^{-9}$ M) that was prepared as a racemate by Schaeffer and Schwender.[10]

The greater potency of dCF and the report that EHNA might have an adverse effect on purine metabolism[11] led to the selection of dCF for clinical studies. Despite promising clinical efficacy,[5-8] dCF causes a high incidence of serious and unpredictable toxicities involving the immune system, kidney, liver, and central nervous system.[12,13] Inhibition of intracellular ADA by dCF is nearly irreversible, with synthesis of new enzyme required for recovery from its effects.[14] It seems likely that toxicities of dCF can be ascribed to this prolonged inhibition of ADA. (±)-EHNA, which is rapidly metabolized[15,16] and has a shorter duration of action, allows faster recovery of the enzyme.

Racemic EHNA was used in early biologic and metabolic studies where enhancement of the activity of *ara*-A was observed.[17,18] The synthesis, by our group[19] and others,[20] of chiral isomers of EHNA allowed identification of the

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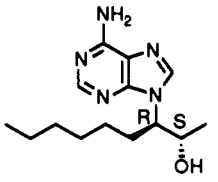
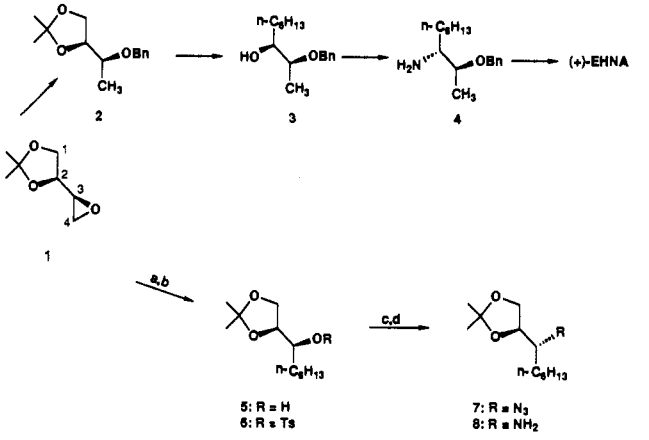


(+)-EHNA

(+)-2S,3R isomer as the most potent ADA inhibitor.[21] (+)-EHNA proved to be the most active isomer in the enhancement of *ara*-A activity against human pancreatic and colon carcinomas.[22] All four stereoisomers caused similar inhibition of purine biosynthesis in sarcoma 180 cells, but these effects were not pronounced below drug concentrations of $1 \times 10^{-5}$ M.[23] The comparable anti-HSV activity of all four isomers suggests that the antiviral activity is not mediated through inhibition of ADA.[24] Recently, an apparent isoenzyme of ADA had been identified, $ADA_2$. Levels of $ADA_2$ are low, but are elevated in the plasma of patients with human immunodeficiency virus (HIV) infections.[25,26] While dCF inhibits both isoenzymes, (+)-EHNA selectively inhibits the predominant ADA activity and has been used in the purification of $ADA_2$.[27]

A shorter-acting ADA inhibitor could permit a controlled duration of chemotherapeutic efficacy while avoiding long-term enzyme inactivation. Attractive targets would be derivatives that are 1–2 orders of magnitude more potent than (+)-EHNA; such derivatives might produce enhanced, but still reversible, ADA inhibition. On the basis of his SAR studies, Schaeffer proposed that the high affinity of EHNA is derived from its ability to bridge the binding region for adenine and sites for the hexyl, hydroxyl, and 1′-methyl groups of the 9-substituent.[28] Subsequent attempts by others to modify the purine moiety of EHNA have met with variable success. When Baker et al. combined the nonyl chain of EHNA with 1,6-dihydro-6-(hydroxymethyl)purine, they observed mutually interfering effects between these high-affinity moieties.[29] On the other hand, certain deaza[30,31] as well as imidazo[32] analogs have shown ADA inhibitory activity comparable to that of EHNA. A recent report on the X-ray crystal structure of ADA with a transition-state analog suggests that the exact mode of binding of ADA inhibitors may soon be revealed.[33] In the present SAR study, C1′ and nor-C1′ derivatives were prepared to explore the 1′-methyl binding pocket. Chirons readily available from L-ascorbic acid[34] facilitated the preparation of these compounds.

**Scheme I**




(a) n-$C_6H_{11}$MgCl, $Et_2O$, -78 °C, 98%; (b) TsCl, Pyr, $CH_2Cl_2$, 84%; (c) $NaN_3$, DMF, 110 °C, 90%; (d) LAH, $Et_2O$, 98%

## Chemistry

In our earlier work[34,35] (+)-EHNA was synthesized starting from epoxide 1 through intermediates 2–4. In this approach C-4 in 1 became a methyl group (C1′) and the nonyl chain was elaborated by *n*-pentylmagnesium chloride addition at C-1. Since the chirality of carbons 2 and 3 in 1 is identical, an inversion of this addition sequence would eventually lead to C1′-substituted (+)-EHNA. Thus, regiospecific opening of epoxide 1 with *n*-pentylmagnesium chloride furnished alcohol **5**. Tosylation of **5** gave **6**, which upon treatment with sodium azide furnished **7**. This was quantitatively reduced to the key amine **8** (Scheme I).

Incorporation of amine **8** into an adenine and subsequent attempts to introduce other functionalities at C1′ are

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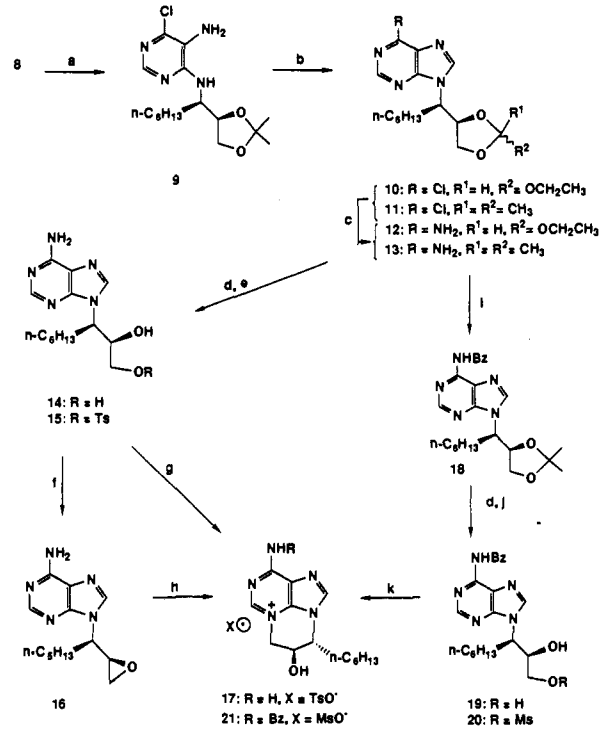
**Scheme II**



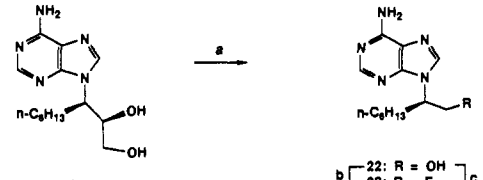
(a) 5-ADCP, n-$Bu_3N$, n-$C_5H_{11}OH$, 120 °C, 56%;(b) $HC(OEt)_3$, $H^+$, 86%; (c) $NH_3$, 80 °C, 92%; (d) $HCO_2H$, $NH_4OH$, 100%; (e) TsCl, Pyr, $CH_2Cl_2$, 84%; (f) n-$Bu_4NCl$, NaOH, TsCl, $CH_2Cl_2$, 22%, (g) $CHCl_3$, 50°C, 73%, (h) rt. (i) BzCl, Pyr, $CH_2Cl_2$, 0 °C, 63%; (j) MsCl, Pyr, $CH_2Cl_2$, 0 °C, 56%; (k) $C_6H_6$, Δ, 95%

outlined in Scheme II. Condensation of **8** with 5-amino-4,6-dichloropyrimidine (ADCP) afforded **9** in good yield, which was converted to two 6-chloropurine derivatives **10** and **11**. The former was formed by an acid-catalyzed exchange between the isopropylidene group and triethyl orthoformate. Amination of this mixture followed by hydrolysis of the protecting groups furnished 1′-hydroxy-(+)-EHNA (**14**) in near-quantitative yield.

Having obtained **14**, our efforts were directed at preparing other 1′-substituted (+)-EHNA derivatives. Of special interest is the 1′-fluoro derivative whose biological activity may provide further information on binding in the methyl pocket. The introduction of a fluorine at C1′ could be accomplished by fluoride-catalyzed opening of the epoxide function in **16**. Thus selective tosylation of the primary hydroxyl group in **14** provided tosylate **15**, which proved to be unstable on standing. It underwent intramolecular cyclization to give the tricyclic compound **17**. This transformation took place in chloroform solution and was monitored by thin-layer chromatography. The $^1H$ NMR spectrum showed the disappearance of the tosylate methyl singlet at δ 2.41 ppm with simultaneous appearance of a methyl singlet at δ 2.31 ppm. Furthermore, the heterocyclic proton resonances at δ 7.88 and 8.15 ppm were shifted downfield to δ 8.22 and 8.44 ppm. Epoxide **16** proved to be equally unstable and could not be used for the introduction of fluorine at the C1′ position.

In an attempt to reduce the nucleophilicity of the N-3 nitrogen, and thus prevent cyclization, N-6 benzolyation was undertaken. When **13** was treated with 1 equiv of benzoyl chloride in pyridine, the corresponding N-6 benzamide **18** was prepared and was hydrolyzed to diol **19**. Surprisingly, conversion of diol **32** to a monotosylate could not be achieved, but mesylate **20** could be easily prepared. Unfortunately, this compound underwent cyclization to the tricyclic product **21** upon standing at room

**Scheme III**



(a) $NaIO_4$, $NaH_2PO_4$, $NaBH_4$, 90%; (b) DAST,$CH_2Cl_2$, 38%; (c) TsCl, Pyr, $CH_2Cl_2$, 50 °C, 96% ; (d) n-$Bu_4NBr$, 78%; n-$Bu_4NCl$, $C_6H_6$, 69%; $NaN_3$, Pyr, 59%.

**Table I.** Inhibition of Adenosine Deaminase

| compound | $K_i$ (μM) | compound | $K_i$ (μM) |
|---|---|---|---|
| (+)-EHNA | $2.0 \times 10^{-3}$ | **23** | 1.6 |
| **14** | $5.6 \times 10^{-2}$ | **25** | $6.0 \times 10^{-1}$ |
| **16** | $8.9 \times 10^{-1}$ | **26** | $3.2 \times 10^{-1}$ |
| **17** | 1.0 | **27** | 1.3 |
| **22** | $4.7 \times 10^{-2}$ | | |

temperature. Other attempts to introduce the fluorine at C1′ included preparation of a fluorinated nonylamine which could be further elaborated into 1′-fluoro(+)-EHNA. Here again, intramolecular nucleophilic displacement of the fluorine atom did take place to form the corresponding cyclic intermediates.

The availability of diol **14** allowed the preparation of C1′-nor derivatives whose biological activity could provide further insight into the nature of the binding site. The modifications are also outlined in Scheme III. Sodium periodate cleavage of **14** followed by reductive workup furnished alcohol **22**. This was converted to the fluoro derivative **23** upon treatment with (diethylamido)sulfur trifluoride (DAST).[36] On the other hand, tosylation of **14** gave **24**, which upon treatment with *n*-$Bu_4NBr$, *N*-$Bu_4$-NCl, and sodium azide furnished the bromo **25**, chloro **26**, and azido **27** derivatives, respectively.

## Results and Discussion

Schaeffer and Schwender studied an extensive series of 9-alkyladenine derivatives to determine structural requirements for high affinity binding to ADA.[10] Our study is an extension of this work to further probe the C2′-hydroxyl and the 1′-methyl binding sites. As shown in Table I, diminished potencies were evident in nor-(+)-EHNA derivatives. The deletion of the 1′-methyl group caused a 23-fold decrease in activity for **22**. A comparable result was reported earlier for the racemate.[37] Derivatives of nor-(+)-EHNA, compounds **23**, **25**, **26**, and **27** had inhibitory activities 7–34-fold lower than nor-(+)-EHNA (**22**) and a 160–800-fold lower than (+)-EHNA itself. The C1′ hydroxy derivative **14** showed a 28-fold decrease in inhibitory activity. Thus, hydroxylation at C1′ (**25**) and removal of the 1′-methyl group (**22**) had similar deleterious effects on activity. This seems to further substantiate the existence of an auxiliary methyl pocket and the importance of the hydroxyl group to binding. Interestingly, the rigid cyclic analog **17** showed higher affinity for the enzyme than the natural nucleoside substrates. The activity of **17**

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was also comparable to that of epoxide **16**, which converted slowly to **17** during testing procedures.

## Experimental Section

Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. The $^1$H NMR spectra were recorded on a Varian EM-390 or a Bruker AM-300 spectrometer. 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. All organic solutions were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. Ratios of chromatography solvents are expressed in v/v. Silica gel (Davison, grade H, 230–425 mesh), suitable for flash column chromatography was purchased from Fisher Scientific. A Chromatotron (centrifugally accelerated, preparative thin-layer, radial chromatograph), Model 7924T was used to complete various separations as indicated. The 1.0- and 2.0-mm plates used were coated with silica gel PF254 containing $CaSO_4$. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

**(2*S*,3*S*)-1,2-*O*-Isopropylidene-1,2,3-nonanetriol (5).** A solution of **1** (0.11 g, 0.56 mmol) in ether (10 mL) was added to a cold (−78 °C) ether solution containing *n*-pentylmagnesium chloride (0.126 mmol) and 0.1 mmol of lithium tetrachlorocuprate with stirring for 3 h. After the mixture warmed to room temperature, stirring was continued for 1 h. The reaction was quenched with a saturated solution of $NH_4Cl$ (100 mL) and extracted with ether. Pure **5** (0.148 g, 96% yield) was obtained by silica gel flash chromatography eluting with EtOAc–hexanes (1:10): $[\alpha]^{22}_D$ −25.23° (*c* 2.67, EtOH); $^1$H NMR ($CDCl_3$) δ 0.87 (t, *J* = 4.5 Hz, 3 H), 1.03–1.61 (m, 16 H), 2.49–2.59 (d, *J* = 4.5 Hz, 1 H, $D_2O$ exchangeable), 3.31–4.09 (m, 4 H). Anal. ($C_{12}H_{24}O_3$) C, H.

**(2*S*,3*S*)-1,2-*O*-Isopropylidene-3-*O*-tosyl-1,2,3-nonanetriol (6).** A solution of **5** (15.0 g, 0.069 mol) in $CH_2Cl_2$ (50 mL) was added to a stirred solution of *p*-toluenesulfonyl chloride (26.5 g, 0.131 mol), anhydrous pyridine (20 mL, 0.250 mol), and $CH_2Cl_2$ (100 mL), and stirring was continued at 50 °C overnight. The solvents were removed, and the residue was dissolved in $CH_2Cl_2$ (100 mL) and washed with water (75 mL). The organic layer was dried and the solvent was removed to give a yellow viscous liquid. Purification by silica gel chromatography, eluting with EtOAc–hexanes (1:10), gave 21.0 g of pure **16** (84% yield): $[\alpha]^{22}_D$ −11.75° (*c* 7.23, EtOH); $^1$H NMR ($CDCl_3$) δ 0.83 (t, *J* = 6 Hz, 3 H), 1.00–1.78 (m, 16 H), 2.43 (s, 3 H), 3.43–4.72 (m, 4 H), 7.29 (d, *J* = 7.5 Hz, 2 H), 7.78 (d, *J* = 7.5 Hz, 2 H). Anal. ($C_{19}H_{30}O_5S$) C, H.

**(2*S*,3*R*)-3-Azido-1,2-*O*-isopropylidene-1,2-nonanediol (7).** Sodium azide (34.0 mg, 0.523 mmol) was added to a stirring solution of **6** (129 mg, 0.349 mmol) in anhydrous DMF (15 mL). After refluxing for 45 min, DMF was removed and pure **7** (80.0 mg, 96% yield) was obtained by silica gel flash chromatography eluting with EtOAc–hexanes (1:26): $[\alpha]^{22}_D$ +32.23° (*c* 1.66, EtOH); $^1$H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6 Hz, 3 H), 1.00–1.69 (m, 16 H), 3.30–3.58 (m, 1 H), 3.76–4.16 (m, 3 H). Anal. ($C_{12}H_{23}N_3O_2$) C, H, N.

**(2*S*,3*R*)-3-Amino-1,2-*O*-isopropylidene-1,2-nonanediol (8).** To a stirred solution of lithium aluminum hydride (LAH, 4.00 g, 0.105 mol) in anhydrous ether (175 mL) was added dropwise a solution of **7** (5.20 g, 0.022 mol) in anhydrous ether (50 mL). The reaction mixture was then heated at reflux for 2 h and cooled to room temperature, and excess LAH was decomposed by the careful successive dropwise addition of water (4 mL), 15% NaOH (4 mL), and water (8 mL). Filtration, drying, and evaporation of the solvent gave a pure yellow liquid **8** (4.52 g, 98% yield): $[\alpha]^{22}_D$ −7.12° (*c* 3.90, EtOH); $^1$H NMR ($CDCl_3$) δ 0.81 (t, *J* = 6 Hz, 3 H), 1.00–1.60 (m, 18 H, 2 $D_2O$ exchangeable), 2.72–2.95 (m, 1 H), 3.72–4.02 (m, 3 H). Anal. ($C_{12}H_{25}NO_2$) C, H, N.

**5-Amino-6-chloro-4-[[1,2(*S*)-(isopropylidenedioxy)-3(*R*)-nonyl]amino]pyrimidine (9).** 5-Amino-4,6-dichloropyrimidine (ADCP, 1.42 g, 8.65 mmol), *n*-$Bu_3N$ (2.1 mL, 8.65 mmol), and **8** (1.69 g, 7.86 mmol) in anhydrous pentanol (50 mL) were heated at reflux for 30 h under $N_2$ atmosphere. Pentanol and *n*-$Bu_3N$ were removed, and the residue was chromatographed over silica gel (EtOAc–hexanes 4:1) to give **9** (2.10 g, 56% yield): $[\alpha]^{22}_D$ +11.03° (*c* 1.68, EtOH); $^1$H NMR ($CDCl_3$) δ 0.80 (t, *J* = 6 Hz, 3 H), 1.06–1.90 (m, 16 H), 3.42–4.50 (m, 6 H, 2 $D_2O$ exchangeable), 4.91–5.01 (d, *J* = 11.4 Hz, 1 H, $D_2O$ exchangeable), 7.93 (s, 1 H). Anal. ($C_{16}H_{27}ClN_4O_2$) C, H, Cl, N.

**6-Chloro-9-[1,2-(ethoxymethylenedioxy)-3(*R*)-nonyl]purine (10). 6-Chloro-9-[1,2(*S*)-(isopropylidenedioxy)-3(*R*)-nonyl]purine (11).** An acidified (concentrated HCl, 0.05 mL) solution of **9** (2.59 g, 7.56 mmol) in triethyl orthoformate (75 mL) was stirred at room temperature for 24 h. The yellow oil obtained after removal of TEOF was purified by silica gel flash chromatography eluting with EtOAc–hexanes (50:3) to provide **10** (0.788 g, 28% yield) and **11** (1.54 g, 58% yield): Compound **10**: $^1$H NMR ($CDCl_3$) δ 0.74 (t, *J* = 6 Hz, 3 H), 0.93–1.60 (m, 11 H), 1.71–2.34 (m, 2 H), 3.49 and 3.51 (q, *J* = 6 Hz, 2 H), 3.73–4.21 (m, 2 H), 4.45–4.83 (m, 2 H), 5.72 and 5.78 (s, 1 H), 8.12 and 8.19 (s, 1 H), 8.68 (s, 1 H). Anal. for **22** ($C_{17}H_{25}ClN_4O_3$) C, H, Cl, N. Compound **11**: $[\alpha]^{22}_D$ +30.46° (*c* 1.65, EtOH); $^1$H NMR ($CDCl_3$) δ 0.75 (t, *J* = 6 Hz, 3 H), 0.95–1.59 (m, 14 H), 1.84–2.29 (m, 2 H), 3.68–4.11 (m, 2 H), 4.33–4.75 (m, 2 H), 8.20 (s, 1 H), 8.65 (s, 1 H). Anal. for **11** ($C_{17}H_{25}ClN_4O_2$) C, H, Cl, N.

**9-[1,2(*S*)-(Isopropylidenedioxy)-3(*R*)-nonyl]adenine (13).** Compound **11** (87.0 mg, 0.247 mmol) was dissolved in liquid ammonia (5 mL) and heated at 90 °C in a steel bomb for 24 h. After cooling, excess ammonia was allowed to evaporate. The residue was taken up in $CH_2Cl_2$ (5 mL) and washed with water (2 mL). The organic layer was dried and pure **13** (76 mg, 90% yield) was obtained as a white solid from hexanes: mp 113 °C; $[\alpha]^{22}_D$ +7.83° (*c* 1.04, EtOH); $^1$H NMR ($CDCl_3$) δ 0.69–1.54 (m, 17 H), 1.87–2.24 (m, 2 H), 3.59–4.07 (m, 2 H), 4.34–4.68 (m, 2 H), 6.52–6.80 (bs, 2 H, $D_2O$ exchangeable), 7.86 (s, 1 H), 8.28 (s, 1 H). Anal. ($C_{17}H_{27}N_5O_2$) C, H, N.

**9-[1,2(*S*)-Dihydroxy-3(*R*)-nonyl]adenine (14).** A solution of **10** and **11** (8.25 g, 10:11, 3:10) in 88% formic acid (25 mL) was stirred at room temperature overnight. The acid was removed and the pH of the solution was adjusted to 8 with 2 N $NH_4OH$ (4 mL). The solution was stirred overnight and filtered through Celite, and the solvent was removed. Crystallization of the product with EtOAc gave pure **14** (7.72 g, 98% yield) as a white powder: mp 161.0 °C; $[\alpha]^{22}_D$ +36.59° (*c* 1.06, EtOH); $^1$H NMR (DMSO-$d_6$) δ 0.65–1.36 (m, 11 H), 1.80–2.22 (m, 2 H), 3.14–3.52 (m, 3 H, 1 H, $D_2O$ exchangeable), 4.43–4.83 (m, 2 H, 1 H, $D_2O$ exchangeable), 5.31 (d, *J* = 6 Hz, 1 H, $D_2O$ exchangeable), 7.10–7.32 (bs, 2 H, $D_2O$ exchangeable), 8.16 (s, 2 H). Anal. ($C_{14}H_{23}N_5O_2$) C, H, N.

**9-[2(*S*)-Hydroxy-1-(tosyloxy)-3(*R*)-nonyl]adenine (15).** To an ice-cold solution of **14** (1.10 g, 3.75 mmol) in anhydrous pyridine (25 mL) was added *p*-toluenesulfonyl chloride (786 mg, 4.12 mmol). After stirring for 2 h, the solvent was removed and intermediate **15** (500 mg, 30% yield) was isolated by silica gel flash chromatography eluting with EtOAc: $^1$H NMR ($CDCl_3$) δ 0.78 (t, *J* = 6 Hz, 3 H), 0.92–1.34 (m, 8 H), 1.50–2.19 (m, 2 H), 2.40 (s, 3 H), 3.89–4.59 (m, 5 H, 1 $D_2O$ exchangeable), 6.20–6.44 (bs, 2 H, $D_2O$ exchangeable), 7.49 (AA′BB′ q, *J* = 9 Hz, 4 H), 7.80 (s, 1 H), 8.14 (s, 1 H). The instability of **15** precluded obtaining correct elemental analysis.

**9-[1,2(*S*)-Epoxy-3(*R*)-nonyl]adenine (16).** A solution of **14** (140 mg, 0.47 mmol) and *n*-$Bu_4NCl$ (13 mg, 0.05 mmol) in $CH_2Cl_2$ (4 mL) was stirred at room temperature for 15 min. A solution of saturated NaOH (2 mL) was then added and stirring was continued for an additional 10 min. While the mixture was stirred vigorously, a solution of tosyl chloride (80 mg, 0.47 mmol) in $CH_2Cl_2$ (2 mL) was added. The reaction was stopped after 2 h, and the residue obtained after evaporation was placed on a silica gel column eluting with MeOH–EtOAc (3:97). The epoxide **16** was obtained (28.5 mg, 22% yield) as an oil: $^1$H NMR ($CDCl_3$) δ 0.70 (t, *J* = 6 Hz, 3 H), 1.15 (bs, 8 H), 1.8–2.15 (m, 2 H), 2.6–2.7 (m, 2 H), 3.10–3.35 (m, 1 H), 4.10–4.44 (m, 1 H), 6.30 (bs, 2 H, $D_2O$ exchangeable), 7.75 (s, 1 H), 8.20 (s, 1 H). The instability of the compound precluded obtaining elemental analysis.

**10-Amino-5,6-dihydro-4(*R*)-hexyl-5(*S*)-hydroxy-4*H*-pyrimido[1,2,3-*cd*]purin-5-ium Tosylate (17).** A solution of **15** (118 mg, 0.264 mmol) in $CHCl_3$ was heated at reflux for 20 h. The solvent was removed and the residue was crystallized from MeOH to give pure **17** as a white powder (86.4 mg, 73.2% yield): mp 198 °C; $^1$H NMR (MeOH-$d_4$) δ 0.85 (t, *J* = 6 Hz, 3 H), 1.04–1.87 (m, 10 H), 2.31 (s, 3 H), 4.23–4.74 (m, 4 H), 7.10 (d, *J* = 9 Hz, 2 H), 7.60 (d, *J* = 9 Hz, 2 H), 8.22 (s, 1 H), 8.44 (s, 1 H). Anal. ($C_{21}H_{29}N_5O_2S$) C, H, N, S.

**$N^6$-Benzoyl-9-[1,2(*S*)-*O*-Isopropylidene-3(*R*)-nonyl]adenine (18).** In a round-bottom flask (100 mL) protected from light, a solution of benzoyl chloride (1.65 mL, 61.8 mmol) in anhydrous pyridine (5.0 mL, 61.8 mmol) was added dropwise to a stirred solution of **13** (4.75 g, 14.2 mmol) in $CH_2Cl_2$ (25 mL). After 48 h the solvent was removed and pure **18** (3.91 g, 63% yield) was obtained by silica gel flash chromatography eluting with EtOAc–hexanes (4:5): $[\alpha]^{25}_D$ +30.28° (*c* 1.66, EtOH); $^1$H NMR ($CDCl_3$) δ 0.61–1.49 (m, 17 H), 1.73–2.23 (m, 2 H), 3.61–4.01 (m, 2 H), 4.30–4.71 (m, 2 H), 7.25–7.55 (m, 3 H, 1 $D_2O$ exchangeable), 7.80–8.10 (m, 3 H), 8.63 (s, 1 H), 9.25 (s, 1 H). Anal. ($C_{24}H_{31}N_5O_3$) C, H, N.

**$N^6$-Benzoyl-9-[1,2(*S*)-dihydroxy-3(*R*)-nonyl]adenine (19).** An acidified (concentrated HCl, 1 mL) solution of **18** (2.66 g, 6.07 mmol) in 95% EtOH (50 mL) was stirred at room temperature for 24 h. The solvent was removed, and pure **19** (1.87 g, 71% yield) and **14** (0.776 g, 22% yield) were obtained by silica gel flash chromatography eluting with EtOAc: mp 158.7–160.0 °C; $[\alpha]^{25}_D$ +28.37° (*c* 1.47, EtOH); $^1$H NMR (DMSO-$d_6$) δ 0.60–1.40 (m, 11 H), 1.77–2.24 (m, 2 H), 3.13–3.45 (m, 2 H, 1 $D_2O$ exchangeable), 3.45–3.65 (m, 1 H), 4.55–4.85 (m, 2 H), 4.95–5.45 (bs, 1 H, $D_2O$ exchangeable), 7.32–7.66 (m, 3 H), 7.85–8.13 (m, 2 H), 8.44 (s, 1 H), 8.65 (s, 1 H). Anal. ($C_{21}H_{27}N_5O_3$) C, H, N.

**$N^6$-Benzoyl-9-[2(*S*)-hydroxy-1-[(methylsulfonyl)oxy]-3-(*R*)-nonyl]adenine (20).** Methanesulfonyl chloride (0.100 mL, 1.36 mmol) was added dropwise to a stirred solution of **19** (541 mg, 1.36 mmol) and anhydrous pyridine (0.5 mL, 38.7 mmol) in anhydrous $CH_2Cl_2$ (25 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h and at room temperature overnight. The solvent was removed and **20** (0.362 g, 56% yield) was obtained by silica gel flash chromatography eluting with EtOAc–hexanes (9:1): $^1$H NMR ($CDCl_3$) δ 0.45–1.41 (m, 11 H), 1.67–2.31 (m, 2 H), 3.10 (s, 3 H), 3.74–4.33 (m, 3 H), 4.53–4.82 (m, 2 H, 1 $D_2O$ exchangeable), 7.29–7.68 (m, 3 H), 7.81–8.12 (m, 3 H), 8.50 (s, 1 H), 8.65 (s, 1 H).

**10-Benzamido-5,6-dihydro-4(*R*)-hexyl-5(*S*)-hydroxy-4*H*-pyrimido[1,2,3-*cd*]purin-5-ium Mesylate (21).** Compound **20** (58.0 mg, 0.122 mmol) in anhydrous benzene (10 mL) was heated at reflux for 1.5 h. The solvent was removed to give pure **21** (55.0 mg, 95% yield): $[\alpha]^{26}_D$ +29.75° (*c* 0.69, EtOH); $^1$H NMR (DMSO-$d_6$) δ 0.85 (t, *J* = 6 Hz, 3 H), 1.00–1.90 (m, 10 H), 2.32 (s, 3 H), 4.40–4.90 (m, 5 H, 1 $D_2O$ exchangeable), 7.35–7.70 (m, 3 H), 7.80–8.13 (m, 2 H), 8.79 (s, 1 H), 9.27 (s, 1 H), 11.88–12.58 (bs, 1 H, $D_2O$ exchangeable). Anal. ($C_{22}H_{29}N_5SO_5$) C, H, N, S.

**9-[1-Hydroxy-2(*R*)-octyl]adenine (22).** To a stirred solution of **14** (947 mg, 3.23 mmol), $NaH_2PO_4 \cdot H_2O$ (70.0 mg, 0.507 mmol) in MeOH–$H_2O$ (3:1, 25 mL) was added $NaIO_4$ (759 mg, 3.55 mmol). The reaction was stirred at room temperature until a heavy white percipitate formed (0.5 h). The reaction mixture was filtered and the filtrate was concentrated to a volume of 15 mL. The solution was then cooled to 0 °C and $NaBH_4$ (0.500 g, 0.0132 mol) was added. The reaction was warmed to room temperature and was stirred for an additional 2 h. The solvent was then removed, and the white crystalline product was washed with small portions of water (3 × 2 mL) to give **22** (0.760 g, 90% yield): mp 150–154 °C; $[\alpha]^{25}_D$ +17.75° (*c* 1.88, EtOH); $^1$H NMR (DMSO-$d_6$) δ 0.65–1.55 (m, 11 H), 1.86–2.20 (m, 2 H), 3.61–4.05 (m, 2 H), 4.38–4.77 (m, 1 H), 4.99–5.22 (bs, 1 H, $D_2O$ exchangeable), 7.09–7.35 (bs, 2 H, $D_2O$ exchangeable), 8.30 (s, 1 H), 8.31 (s, 1 H). Anal. ($C_{13}H_{21}N_5O$) C, H, N.

**9-[1-Fluoro-2(*R*)-octyl]adenine (23).** To a cold (−78 °C) solution of (diethylamido)sulfur trifluoride (DAST, 0.15 mL, 1.14 mmol) in $CH_2Cl_2$ (3 mL) was slowly added a solution of **22** (113 mg, 0.430 mmol) in anhydrous $CH_2Cl_2$ (3 mL), and stirring was continued for 1 h. After stirring for 1 h at room temperature, excess DAST was decomposed by the careful addition of cold saturated solution of $NaHCO_3$ (5 mL). The crude product was extracted with $CH_2Cl_2$ (10 mL) and pure **23** (44.0 mg, 38% yield) as a white powder was obtained by silica gel flash chromatography eluting with EtOAc: mp 117–118 °C; $[\alpha]^{26}_D$ +26.77° (*c* 0.79, EtOH); $^1$H NMR ($CDCl_3$) δ 0.85 (t, *J* = 6.6 Hz, 3 H), 1.23–1.33 (m, 8 H), 1.97–2.14 (m, 2 H), 4.58–4.96 (m, 3 H), 6.40 (bs, 2 H, $D_2O$ exchangeable), 7.92 (s, 1 H), 8.35 (s, 1 H). Anal. ($C_{13}H_{20}N_5F$) C, H, F, N.

**9-[1-(Tosyloxy)-2(*R*)-octyl]adenine (24).** This compound was prepared from **19** as indicated previously for **6** in 96% yield: $[\alpha]^{25}_D$ +5.32° (*c* 2.57, EtOH); $^1$H NMR ($CDCl_3$) δ 0.80 (t, *J* = 6 Hz, 3 H), 1.03–1.45 (m, 8 H), 1.83–2.18 (m, 2 H), 2.40 (s, 3 H), 4.22–4.84 (m, 3 H), 6.07–6.33 (bs, 2 H, $D_2O$ exchangeable), 7.10 (d, *J* = 7.5 Hz, 2 H), 7.50 (d, *J* = 7.5 Hz, 2 H), 7.73 (s, 1 H), 8.14 (s, 1 H). Anal. ($C_{20}H_{27}N_5O_3S$) C, H, N.

**9-[1-Bromo-2(*R*)-octyl]adenine (25).** An anhydrous benzene solution (25 mL) of *n*-$Bu_4NBr$ (0.325 g, 1.01 mmol) and **24** (85.0 mg, 0.204 mmol) was heated at reflux for 2.5 h. The solvent was removed and pure **25** (51.9 mg, 78% yield) as a white powder was obtained by silica gel flash chromatography eluting with EtOAc: mp 85–88 °C; $[\alpha]^{27}_D$ +13.16° (*c* 0.77, EtOH); $^1$H NMR ($CDCl_3$) δ 0.80 (t, *J* = 6 Hz, 3 H), 1.01–1.47 (m, 8 H), 1.89–2.32 (m, 2 H), 3.63–4.19 (m, 2 H), 4.49–4.87 (m, 1 H), 6.00–6.29 (bs, 2 H, $D_2O$ exchangeable), 7.80 (s, 1 H), 8.29 (s, 1 H). Anal. ($C_{13}H_{20}BrN_5$) C, H, Br, N.

**9-[1-Chloro-2(*R*)-octyl]adenine (26).** An anhydrous benzene solution (25 mL) of *n*-$Bu_4NCl$ (0.22 g, 0.791 mmol) and **24** (0.11 g, 0.263 mmol) was heated at reflux for 2 h. The solvent was removed and pure **26** (51.2 mg, 69% yield) as light yellow crystals was obtained by silica gel chromatography eluting with EtOAc: mp 78–79 °C, $[\alpha]^{27}_D$ +21.26° (*c* 1.93, EtOH); $^1$H NMR ($CDCl_3$) δ 0.79 (t, *J* = 6 Hz, 3 H), 0.96–1.48 (m, 8 H), 2.82–2.26 (m, 2 H), 3.72–4.20 (m, 2 H), 4.52–4.84 (m, 1 H), 6.40–6.66 (bs, 2 H, $D_2O$ exchangeable), 7.81 (s, 1 H), 8.27 (s, 1 H). Anal. ($C_{13}H_{20}ClN_5$) C, H, Cl, N.

**9-[1-Azido-2(*R*)-octyl]adenine (27).** A solution of **24** (0.11 g, 0.262 mmol), $NaN_3$ (35.0 mg, 0.538 mmol), and pyridine (0.03 mL, 0.538 mmol) in DMF (13 mL) was heated at reflux for 15 h. The solvent was removed and the resulting mixture was dissolved in $CH_2Cl_2$ (15 mL) and washed with water (10 mL). The solvent was removed and pure **27** (44.5 mg, 59% yield) was obtained by silica gel chromatography eluting with EtOAc: $[\alpha]^{25}_D$ +21.74° (*c* 1.38, EtOH); $^1$H NMR ($CDCl_3$) δ 0.74 (t, *J* = 6 Hz, 3 H), 1.02–1.50 (m, 8 H), 1.80–2.30 (m, 2 H), 3.58–4.13 (m, 2 H), 4.43–4.78 (m, 1 H), 6.33–6.65 (bs, 2 H, $D_2O$ exchangeable), 7.85 (s, 1 H), 8.33 (s, 1 H). Anal. ($C_{13}H_{20}N_8$) C, H, N.

**Biological Evaluation.** The activity of calf intestinal ADA (Type III, Sigma Chemical Co.) was measured at 30 °C by direct spectrophotometric assays at 265 nm,[38,39] using a Gilford Model 240 spectrophotometer. The 1-mL reaction mixtures contained 0.003 unit of enzyme, 10–80 μM adenosine, and 50 mM potassium phosphate, pH 7.4. Inhibition constants below 900 nM were determined from replots of the slopes of double-reciprocal plots at multiple inhibitor concentrations. Micromolar $K_i$ values were calculated from apparent $K_i$ values obtained from plots of 1/velocity vs inhibitor concentration by the equation, $K_i^{app} = K_i(1 + [S]/K_m)$. Inhibition was assumed to be competitive and adenosine concentration was fixed at 24 μM, approximately the $K_m$ value.

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