Notes

Adenosine Deaminase Inhibitors: Synthesis and Structure–Activity Relationships of 2-Hydroxy-3-nonyl Derivatives of Azoles†

Gloria Cristalli,* Alessandra Eleuteri, Rosaria Volpini, Sauro Vittori, Emido Camaioni, and Giulio Lupidi‡

Dipartimento di Scienze Chimiche and Dipartimento di Biologia Cellulare, Università di Camerino, 62032 Camerino, Italy

Received June 30, 1993•

A series of *erythro*-1-(2-hydroxy-3-nonyl)azole derivatives have been synthesized and evaluated for adenosine deaminase (ADA) inhibitory activity, in order to introduce simplifications in the ADA inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, **1a**). The synthesis of most of the reported compounds was achieved by reaction of 2-bromo-3-nonanone with the suitable azole followed by reduction of the carbonyl group to give a diastereoisomeric mixture of N-substituted (2-hydroxy-3-nonyl)azoles. Separation of diastereoisomers was achieved by HPLC or by preparative TLC plates. The results of the enzymatic test indicate that the nitrogen in the 3-position, and secondly, the nitrogen in the 5-position are very important for the interaction of the azole ring with the inhibitory site on the enzyme. In fact, the pyrazole and the 2-substituted 1,2,3-triazole derivatives (**10** and **15**, respectively) are nearly inactive, whereas the *erythro*-1-(2-hydroxy-3-nonyl)-1,2,4-triazole (**18e**) was the most potent ADA inhibitor in the series with K_i = 0.3 μM.

Deamination of adenosine and 2′-deoxyadenosine to form inosine and 2′-deoxyinosine is catalyzed by adenosine deaminase (ADA).

This enzyme is present in all mammalian cells and plays a central role also in the differentiation and maturation of lymphoid system cells. Hereditary adenosine deaminase deficiency causes severe combined immunodeficiency disease (ADA-SCID), in which both B-cell and T-cell development is impaired.[1,2] Enzyme abnormalities have been reported also in some leukaemia diseases[3] and in acquired immunodeficiency syndrome (AIDS).[3,4] It has been suggested that modulating ADA activity may be a target for chemotheraphy. Therefore, ADA inhibitors may be used both as drugs and as codrugs in combination with certain anticancer or antiviral agents which are adenosine analogues.[5-7]

The function of ADA is also critical in controlling the effects of adenosine in other systems. Adenosine is an endogenous antihypoxic and anticonvulsant and a modulator of platelet aggregation, lipolysis, glycogenolysis, blood flow, and neurotransmission.[8,9] Therefore, modulation of adenosine deaminase with the use of highly specific inhibitors might modify the action of endogenous adenosine under various physiological and pathological conditions.[10-12]

All these aspects have greatly stimulated our interest in synthesizing new enzyme inhibitors, with the aim of clarifying the requirements of the ADA inhibitory site and possibly providing new therapeutic approaches. Among the known ADA inhibitors, *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA, **1a**), a semi-tight-binding inhibitor (K_i = 0.007 μM), is advocated as a possible inhibitor of choice for use with nucleosides, which are ADA substrates, and was preferred to coformycin and deoxycoformycin, two extremely potent naturally occurring inhibitors (K_i = 0.01–0.001 nM).[13] In order to investigate which structural parameters in the purine moiety of EHNA were critical for inhibitory activity, we synthesized and tested a series of deaza analogues of EHNA.[14-17]

We demonstrated that isosteric monosubstitution of the pyrimidine nitrogens by carbons can be tolerated at the enzymatic binding site. In fact, 3-deazaEHNA (K_i = 0.01 μM) was found to have an inhibitory activity comparable to EHNA itself, and 1-deazaEHNA (K_i = 0.16 μM), though less potent, is a good inhibitor. On the other hand, substitution of a methine group for the nitrogen atom in the 7-position of the purine moiety of EHNA produced a dramatic drop in the inhibitory activity (7-deazaEHNA, K_i = 400 μM). Also the substitution of both pyrimidine nitrogens by carbons brought about a considerable reduction of activity (1,3-dideazaEHNA, K_i = 71 μM).

In order to introduce additional simplifications to the EHNA chemical structure, a series of *erythro*-1-(2-hydroxy-3-nonyl)imidazole derivatives have been synthesized and tested.[17] As shown in Table I, opening the pyrimidine or pyridine ring of EHNA led to compounds which are still ADA inhibitors. The amino group at C-4 of the most potent compound *erythro*-1-(2-hydroxy-3-nonyl)imidazole-4-carboxamide (**1b**, K_i = 0.035 μM) provided potential donor and acceptor sites for hydrogen bonding. Lack of one of these sites could account for the order of potency of all compounds examined in this series.[17,18] The finding that *erythro*-1-(2-hydroxy-3-nonyl)imidazole itself was a good ADA inhibitor (**6**, K_i = 0.90 μM) prompted us to couple the 2-hydroxy-3-nonyl chain to other aromatic azole systems, i.e. pyrazole, 1,2,3-triazole, 1,2,4-triazole, and 1,2,3,4-tetrazole.

Chemistry

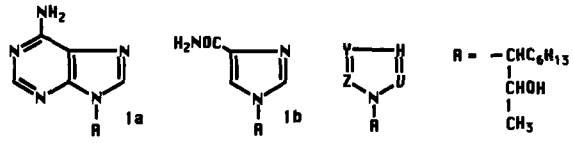
Most of compounds reported in this paper were synthesized by reacting 3-bromo-2-nonanone (**3**) with the appropriate azole, followed by reduction of the carbonyl group with sodium borohydride.

Preparation of compound **3** was accomplished by treatment of 2-nonanone (**2**) with the commercially

† Dedicated to Prof. Leroy B. Townsend in occasion of his 60th birthday.
‡ Dipartimento di Biologia Cellulare.
• Abstract published in *Advance ACS Abstracts*, November 15, 1993.

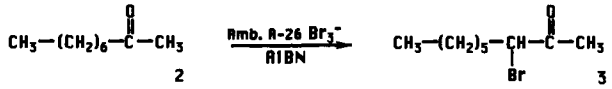
0022-2623/94/1837-0201$04.50/0

Table I. ADA Inhibitory Activity of 2-Hydroxy-3-nonyl Derivatives of Azoles

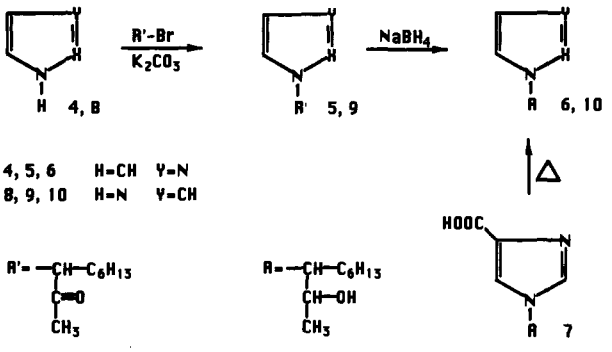


no.	V	X	Y	Z	K_i, μM
1a					0.007
1b					0.035
6e	CH	N	CH	CH	0.7
6t	CH	N	CH	CH	1.8
10	N	CH	CH	CH	>1000
13e	N	N	CH	CH	24.0
13t	N	N	CH	CH	100
15	N	CH	CH	N	>400
18e	CH	N	CH	N	0.3
18t	CH	N	CH	N	1.0
20e	CH	N	N	CH	26.0
23e	CH	N	N	N	0.6
23t	CH	N	N	N	15.0
25e	N	N	CH	N	9.6
25t	N	N	CH	N	>1000

Scheme I



Scheme II



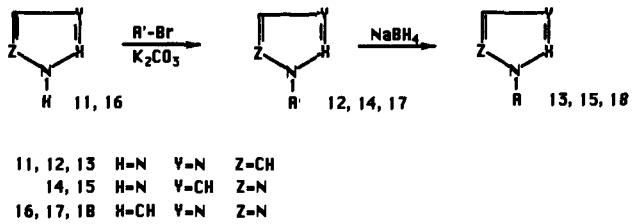
available anion-exchange resin Amberlyst A-26, perbromide form (Fluka), in the presence of methyloxirane and of the free-radical initiator azobisisobutyronitrile (AIBN).[19] The conditions described by Bongini et al. for bromination of 2-octanone (refluxing in hexane for 30 min) gave only polybrominated derivatives in the case of 2-nonanone. Alternatively, the reaction mixture was heated at 40 °C for 5 h to obtain a 38% yield of 3-bromo-2-nonanone (3) (Scheme I).

Reaction at room temperature of 3 with pyrazole (8), 1,2,3-triazole (11), 1,2,4-triazole (16), 1,2,3,4-tetrazole (21), in tetrahydrofuran–dimethylformamide (1:1) in the presence of potassium carbonate gave 1-(2-keto-3-nonyl)-pyrazole) (9), 1-(2-keto-3-nonyl)-1,2,3-triazole (12), and 2-(2-keto-3-nonyl)-1,2,3-triazole (14), 1-(2-keto-3-nonyl)-1,2,4-triazole (17), 1-(2-keto-3-nonyl)-1,2,3,4-tetrazole (22) and 2-(2-keto-3-nonyl)-1,2,3,4-tetrazole (24), respectively (Schemes II–IV).

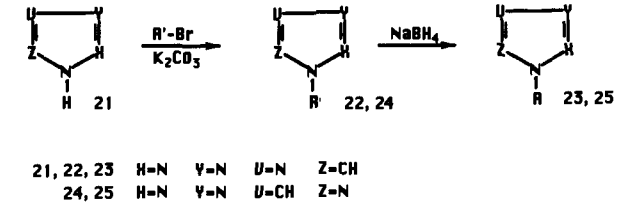
Reaction of 3 with 1,2,3-triazole (11) gave a mixture of the two N-1 and N-2 substituted isomers, compounds 12 and 14, respectively (Scheme III).

The site of alkylation was established on the basis of ^{1}H NMR spectra of the corresponding hydroxy derivatives 1-(*erythro*-2-hydroxy-3-nonyl)-1,2,3-triazole (13e) and 2-(*erythro*-2-hydroxy-3-nonyl)-1,2,3-triazole (15e). Compound 13e exhibited two doublets at δ 7.73 and 8.13, respectively: the downfield signal was assigned to H-5 and the upfield one to H-4, according to the spectral date in DMSO-d_6 reported in literature for the corresponding N^1-methyl derivative.[20] On the other hand, the symmetric compound 15e exhibited in the aromatic field a doublet with coupling constant of 1.2 Hz, assigned to H-4 and H-5 protons.

Scheme III



Scheme IV



Reaction of 3 with 1,2,4-triazole (16) afforded only the 1-substituted isomer 17: the alkylation site was confirmed by ^{1}H NMR spectrum which exhibited two singlets at δ 8.06 and 8.66, instead of a two-proton singlet as expected in the case of the symmetric isomer 4-(2-keto-3-nonyl)-1,2,4-triazole (Scheme III). The synthesis of 4-(*erythro*-2-hydroxy-3-nonyl)-1,2,4-triazole (20) was alternatively accomplished by refluxing formylhydrazine and triethyl orthoformate, and then adding *erythro*-3-amino-2-nonanol (19)[21] to the cooled and acidified mixture: the ^{1}H NMR spectrum of compound 20 exhibited a two-proton singlet at δ 8.49, corresponding to the symmetric H-2 and H-5 protons (Scheme III).

Coupling of 3 with 1,2,3,4-tetrazole (21) gave alkylation both in N-1, affording 1-(2-keto-3-nonyl)-1,2,3,4-tetrazole (22), and in N-2, yielding 2-(2-keto-3-nonyl)-1,2,3,4-tetrazole (24) (Scheme IV). The assignment of the alkylation site was made for the alcohols 1-(*erythro*-2-hydroxy-3-nonyl)-1,2,3,4-tetrazole (23e) and 2-(*erythro*-2-hydroxy-3-nonyl)-1,2,3,4-tetrazole (25e) and was based on the ^{1}H NMR spectral data in DMSO-d_6 reported in literature for the corresponding *N*-methyl derivatives.[20] The structural assignment was unambiguously confirmed by the recent 2D ^{1}H, ^{13}C-correlation spectroscopy technique. In fact, according to the literature, the signal of the H-5 proton was observed downfield when the alkylation occurred at N-1 whereas the same signal appeared upfield when the alkylation occurred at N-2 (**23e** δ = 9.43 vs **25e** δ = 9.00). Moreover, the 2D ^{1}H,^{13}C-correlation spectroscopy exhibited a long-range coupling between H-3′ and C-5 only in the case of **23e**, clearly indicating that in this derivative the ribosylation occurred at N-1.

Coupling of 3 with imidazole (4) at room temperature gave no reaction, and 1-(2-keto-3-nonyl)imidazole (5) was

obtained by raising the temperature to 70 °C. Also in the case of the imidazole derivative the signal of the H-5 proton appeared downfield (δ = 7.17) than that of the H-4 one (δ = 6.97), according to the ^{1}H NMR data reported in literature for the 1-methylimidazole (Scheme II).[20]

Reduction of compounds **5**, **9**, **12**, **14**, **17**, **11**, **24** with $NaBH_4$ in methanol at room temperature provided 1-(2-hydroxy-3-nonyl)imidazole (**6**), 1-(2-hydroxy-3-nonyl)-pyrazole (**10**), 1-(2-hydroxy-3-nonyl)-1,2,3-triazole (**13**), 2-(2-hydroxy-3-nonyl)-1,2,3-triazole (**15**), 1-(2-hydroxy-3-nonyl)-1,2,4-triazole (**18**), 1-(2-hydroxy-3-nonyl)-1,2,3,4-tetrazole (**23**), and 2-(2-hydroxy-3-nonyl)-1,2,3,4-tetrazole (**25**), respectively (Scheme II–IV). All the reduction products were obtained as 1:1 racemic mixture of two diastereoisomers. Separation of diastereoisomers was achieved by HPLC (**6** and **23**) or by preparative TLC plates (**13**, **18**, **25**). Compounds **10** and **15**, which were almost inactive as inhibitors of ADA, were tested as racemic mixture.

The imidazole derivative **6** was also obtained by pyrolitic decarboxylation of *erythro*-1-(2-hydroxy-3-nonyl)imidazole-4-carboxylic acid (**7**)[17] (Scheme II).

The diastereoisomeric configuration of 2-hydroxy-3-nonyl derivatives was assigned by ^{1}H NMR data, in comparison with the imidazole derivative **6** which presents unambiguously the *erythro* configuration, since it was obtained by decarboxylation of the *erythro* derivative **7**. Accordingly, the azole ring and the OH protons of the *erythro* diastereomers always appeared downfield from those of the corresponding *threo* diastereomers, and the CH_3-1 protons of the *erythro* diastereomers appeared upfield from those observed for the *threo* diastereomers.

Biological Evaluation and Discussion

The synthesized (2-hydroxy-3-nonyl)azoles were tested as inhibitors of adenosine deaminase from calf intestine and the results are reported in Table I. The most potent ADA inhibitor in the series resulted to be *erythro*-1-(2-hydroxy-3-nonyl)-1,2,4-triazole (**18e**, K_i = 0.3 μM). The *erythro* isomers were always more potent than the corresponding *threo* isomers, according to the profile of EHNA itself. The difference in potency between the two diastereoisomers is higher in the case of tetrazole rings (**23e**, K_i = 0.6 μM vs **23t**, K_i = 15 μM, and **25e**, K_i = 9.6 μM vs **25t**, K_i > 400 μM) in keeping with the increased interaction of nitrogen atoms with the inhibitory site.

Structure–activity relationships of ADA inhibitory potency of azole derivatives compared to imidazole showed that the presence of a nitrogen in the 3-position of the azole ring is crucial for the activity; in fact both the pyrazole (**10**) and the 2-substituted 1,2,3-triazole derivative (**15**) were almost inactive as ADA inhibitors compared to the imidazole derivative (**10**, K_i > 1000 μM, and **15** K_i > 400 μM vs **6e**, K_i = 0.7 μM). The introduction of a nitrogen in the 2- or 4-position of the imidazole ring produced a decrease in activity of more than 1 order of magnitude (**13e**, K_i = 24 μM and **20e**, K_i = 26 μM, respectively).

The presence of a nitrogen in the 5-position produced an increase in inhibitory activity as shown by the 1,2,4-triazole derivative, **18e** compared to the imidazole derivative **6e** (K_i = 0.3 μM vs K_i = 0.7 μM) and by the tetrazole **25e** (K_i = 9.6 μM) compared to the 1,2,3-triazole derivative **13e** (K_i = 24 μM). The increase in inhibitory potency is even more dramatic when a nitrogen in the 4-position is present in the azole ring (**23e**, K_i = 0.6 μM vs **20e**, K_i = 26 μM). These results indicate that the nitrogen in the 3-position, and secondly the nitrogen in the 5-position, are very important for the interaction of the azole ring with the inhibitory site on the enzyme. In the case the electron density on the nitrogen and in the 3-position is lowered, the inhibitory activity is reduced. On the other hand, a lower electron density on N-4 of the azole ring seems to be required to increase the potency. Moreover, these results together with our previous finding that the presence of potential donor and acceptor sites for hydrogen bonding in the 4-position of the imidazole ring is required to optimize the inhibitory activity, as shown by *erythro*-1-(2-hydroxy-3-nonyl)imidazole-4-carboxamide (**1b**, K_i = 0.035 mM), prompt us to undertake next the synthesis of derivatives of **1b** itself with electron-withdrawing groups in the 5-position or with electron-donor groups in the 2-position.

Experimental Section

Chemistry. Melting points were determined with a Buchi apparatus and are uncorrected, ^{1}H NMR spectra were obtained with a Varian VX 300 MHz instrument. High-performance liquid chromatography (HPLC) was performed on a Hewlett-Packard series 1050 using a Supelcosil SPLC-Si column (25 × 10 mm, 5-μm particle size, 100-Å pore diameter) and a HP 1047-Å RI detector. Analytical TLC was carried out on precoated TLC plates with silica gel 60 F-254 (Merck). Preparative TLC was performed on precoated plates with silica gel 60 F-254 (Whatman PK6F). For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are indicated by atomic symbols and are within ±0.4% of theoretical values.

3-Bromo-2-nonanone (3). To an ice-cooled solution of 6 g (42.2 mmol) of 2-nonanone (**2**) in *n*-hexane was added 36 g of Amberlyst A-26, perbromide form, 2.4 mL of methyloxirane, and 100 mg of azobisisobutyronitrile (AIBN).[19] The reaction mixture was heated at 40 °C for 5 h and then filtered and concentrated in vacuo. The residue was flash chromatographed on a silica gel column, eluting with c-C_6H_{12}–C_6H_6–CH_2Cl_2 (93:5:2) to give 3.5 g (38%) of **3** as a chromatographically pure oil: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, CH_3-9), 1.31 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.96 (m, 2H, CH_2-4), 2.37 (s, 3H, CH_3-1), 4.23 (m, 1H, CH-3). Anal. ($C_9H_{17}BrO$) C, H.

General Method for the Preparation of *N*-(2-Keto-3-nonyl)azoles (Procedure I). To 4.0 mmol of the appropriate azole was added 1.0 g (4.8 mmol) of **3** in 10 mL of THF–DMF (1:1) and 690 mg (5.0 mmol) of K_2CO_3. The reaction mixture was stirred at room temperature for 20 h and then filtered and concentrated in vacuo (oil pump). The residue was chromatographed on a silica gel column, eluting with a suitable mixture of solvents.

General Method for the Preparation of *N*-(2-Hydroxy-3-nonyl)azoles (Procedure II). To an ice-cooled solution of 1 mmol of the appropriate *N*-(2-keto-3-nonyl)azole in 10 mL of methanol was added portionwise 135 mg (3.5 mmol) of 98% $NaBH_4$ while maintaining the pH between 5 and 6 by adding glacial acetic acid. The reaction mixture was stirred at room temperature for 20 h and then concentrated in vacuo. The residue was neutralized with saturated $NaHCO_3$ solution and extracted with chloroform. The combined extracts were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was flash chromatographed on a silica gel column, eluting with a suitable mixture of solvents.

1-(2-Keto-3-nonyl)imidazole (5). The title compound was prepared according to the general procedure I. Purification by silica gel column chromatography eluting with CH_2Cl_2–MeOH (97:3) gave 63% yield: ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, CH_3-9), 1.00 (m, 2H, CH_2-8), 1.22 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 1.87 and 2.06 (m, 2H, CH_2-4), 2.09 (s, 3H, CH_3-1), 5.02 (m, 1H, CH-3), 6.97 (s, 1H, H-4), 7.17 (s, 1H, H-5), 7.67 (s, 1H, H-2). Anal. ($C_{12}H_{20}N_2O$) C, H, N.

1-(2-Hydroxy-3-nonyl)imidazole (6). Method A. The title compound was prepared according to the general procedure II. Purification by silica gel flash chromatography eluting with CH_2-

Cl_2–MeOH (99:1) gave 81% yield. The mixture of two diastereoisomers were separated by preparative HPLC eluting with AcOEt–MeOH (98:2) (2 mL/min).

erythro-**6e**: ^{1}H NMR (DMSO-d_6) δ 0.84 (m, 6H, CH_3-1 and CH_3-9), 1.20 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.74 and 1.96 (m, 1H each, CH_2-4), 3.76 (m, 2H, CH-3 and CH-2), 6.89 (s, 1H, H-4), 7.16 (s, 1H, H-5), 7.62 (s, 1H, H-2). Anal. ($C_{12}H_{22}N_2O$) C, H, N.

threo-**6t**: ^{1}H NMR (DMSO-d_6) δ 0.86 (m, 6H, CH_3-1 and CH_3-9), 1.21 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.74 (m, 2H, CH_2-4), 3.85 (m, 2H, CH-3 and CH-2), 6.87 (s, 1H, H-4), 7.13 (s, 1H, H-5), 7.57 (s, 1H, H-2). Anal. ($C_{12}H_{22}N_2O$) C, H, N.

Method B. The title compound was prepared by heating 200 mg (0.79 mmol) of *erythro*-1-(2-hydroxy-3-nonyl)imidazole-4-carboxylic acid (**7**)[17] at 180 °C under vacuum (water pump) for 4 h. The residue was partitioned between 0.2 N NaOH and chloroform, and the organic layer was separated, washed with water, and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed over silica gel eluting with $CHCl_3$–MeOH (95:5) to give 50 mg (30%) of *erythro*-1-(2-hydroxy-3-nonyl)imidazole (**6e**) as a chromatographically pure oil.

1-(2-Keto-3-nonyl)pyrazole (9). The title compound was prepared according to the general procedure I. Purification by silica gel column chromatography eluting with c-C_6H_{12}–AcOEt (90:10) gave 40% yield: ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H, CH_3-9), 0.98 (m, 2H, CH_2-8), 1.20 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 1.87 (s, 3H, CH_3-1), 1.97 (m, 2H, CH_2-4), 5.02 (m, 1H, CH-3), 6.33 (m, 1H, H-4), 7.53 (d, 1H, $J_{5,4}$ = 2.0 Hz, H-5), 7.85 (d, 1H, $J_{3,4}$ = 2.2 Hz, H-3). Anal. ($C_{12}H_{20}N_2O$) C, H, N.

1-(2-Hydroxy-3-nonyl)pyrazole (10). The title compound was prepared according to the general procedure II. Purification by silica gel column chromatography eluting with $CHCl_3$ gave 50% yield: ^{1}H NMR (DMSO-d_6) δ 0.71 and 0.90 (d, 3H, CH_3-1), 0.80 (t, 3H, CH_3-9), 1.15 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.83 (m, 2H, CH_2-4), 3.79 (m, 1H, CH-3), 4.01 (m, 1H, CH-2), 6.18 (m, 1H, H-4), 7.37 and 7.40 (d, 1H, $J_{5,4}$ = 1.8 Hz, H-5), 7.63 and 7.66 (d, 1H, $J_{3,4}$ = 1.8 Hz, H-3). Anal. ($C_{12}H_{22}N_2O$) C, H, N.

1-(2-Keto-3-nonyl)-1,2,3-triazole (12) and 2-(2-keto-3-nonyl)-1,2,3-triazole (14). The title compounds were prepared according to the general procedure I. Purification by silica gel column flash chromatography eluting with c-C_6H_{12}–$CHCl_3$ (70:30) gave 52% yield of the faster moving **14** and 42% yield of **12**.

12: ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, CH_3-9), 0.97 (m, 2H, CH_2-8), 1.11 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 1.88 (s, 3H, CH_3-1), 2.08 (m, 2H, CH_2-4), 5.22 (m, 1H, CH-3), 7.89 (s, 2H, H-4 and H-5); ^{1}H NMR ($CDCl_3$) δ 7.67 and 7.78 (s, 1H each, H-4 and H-5). Anal. ($C_{11}H_{19}N_3O$) C, H, N.

14: ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, CH_3-9), 0.97 (m, 2H, CH_2-8), 1.23 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 1.89 (s, 3H, CH_3-1), 2.08 (m, 2H, CH_2-4), 5.43 (m, 1H, CH-3), 7.88 (s, 2H, H-4 and H-5); ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 2H, H-4 and H-5). Anal. ($C_{11}H_{19}N_3O$) C, H, N.

1-(2-Hydroxy-3-nonyl)-1,2,3-triazole (13). The title compound was prepared according to the general procedure II. Purification by silica gel column flash chromatography eluting with CH_2Cl_2–MeOH (99:1) gave 82% yield of **13** as a mixture of two diastereoisomers which were separated by preparative TLC plate eluting with c-C_6H_{12}–AcOEt–CH_3CN (40:30:30).

erythro-**13e**: ^{1}H NMR (DMSO-d_6) δ 0.84 (m, 6H, CH_3-1 and CH_3-9), 1.21 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.97 (m, 2H, CH_2-4), 3.88 (m, 1H, CH-3), 4.33 (m, 1H, CH-2), 7.73 (d, 1H, $J_{4,5}$ = 1.1 Hz, H-4), 8.13 (d, 1H, $J_{5,4}$ = 1.1 Hz, H-5). Anal. ($C_{11}H_{21}N_3O$) C, H, N.

threo-**13t**: ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, CH_3-9), 0.93 (d, 3H, CH_3-1), 1.18 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.86 (m, 2H, CH_2-4), 3.95 (m, 1H, CH-3), 4.43 (m, 1H, CH-2), 7.71 (d, 1H, $J_{4,5}$ = 1.1 Hz, H-4), 8.03 (d, 1H, $J_{5,4}$ = 1.1 Hz, H-5). Anal. ($C_{11}H_{21}N_3O$) C, H, N.

2-(2-Hydroxy-3-nonyl)-1,2,3-triazole (15). The title compound was prepared according to the general procedure II. Purification by silica gel column flash chromatography eluting with c-C_6H_{12}–$CHCl_3$–C_6H_6 (50:25:25) gave 84% yield of **15** as a mixture of two diastereoisomers; ^{1}H NMR (DMSO-d_6) δ 0.78 (t, 3H, CH_3-9), 0.69 and 1.04 (d, 3H, CH_3-1), 1.18 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.91 (m, 2H, CH_2-4), 3.83 and 3.93 (m, 1H, CH-3), 4.21 and 4.36 (m, 1H, CH-2), 7.71 and 7.74 (d, 2H, $J_{4,5}$ = 1.2 Hz, H-4 and H-5). Anal. ($C_{11}H_{21}N_3O$) C, H, N.

1-(2-Keto-3-nonyl)-1,2,4-triazole (17). The title compound was prepared according to the general procedure I. Purification by silica gel column flash chromatography eluting with $CHCl_3$–c-C_6H_{12} (50:50) gave 82% yield: ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, CH_3-9), 0.98 (m, 2H, CH_2-8), 1.22 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 2.02 (s, 3H, CH_3-1), 2.03 (m, 2H, CH_2-4), 5.25 (m, 1H, CH-3), 8.06 (s, 1H, H-3), 8.66 (s, 1H, H-5). Anal. ($C_{11}H_{19}N_3O$) C, H, N.

1-(2-Hydroxy-3-nonyl)-1,2,4-triazole (18). The title compound was prepared according to the general procedure II. Purification by silica gel column flash chromatography eluting with $CHCl_3$–MeOH (98:2) gave 72% yield of **18** as a mixture of two diastereoisomers which were separated by preparative TLC plate eluting with AcOEt–c-C_6H_{12} (90:10).

erythro-**18e**: ^{1}H NMR (DMSO-d_6) δ 0.82 (m, 6H, CH_3-9 and CH_3-1), 1.19 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.93 (m, 2H, CH_2-4), 3.84 (m, 1H, CH-3), 4.08 (m, 1H, CH-2), 7.97 (s, 1H, H-3), 8.52 (s, 1H, H-5). Anal. ($C_{11}H_{21}N_3O$) C, H, N.

threo-**18t**: ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3-9), 1.02 (d, 3H, J = 6.3 Hz, CH_3-1), 1.21 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.83 (m, 2H, CH_2-4), 3.87 (m, 1H, CH-3), 4.15 (m, 1H, CH-2), 7.94 (s, 1H, H-3), 8.44 (s, 1H, H-5). Anal. ($C_{11}H_{21}N_3O$) C, H, N.

4-(*erythro*-2-Hydroxy-3-nonyl)-1,2,4-triazole (20). To 200 mg (3.33 mmol) of formylhydrazine was added 530 mg (0.66 mL, 3.93 mmol) of triethyl orthoformate, and the mixture was heated under reflux for 1 h. To the cooled mixture was added 626 mg (3.93 mmol) of *erythro*-3-amino-2-nonanol (**19**)[21] and 12 mL of 0.1 N hydrochloric acid, and the solution was stirred at room temperature overnight. The solvent was evaporated, and the residue was chromatographed on a silica gel column. Elution with EtOAc–MeOH (91:9) yielded 140 mg (20%) of **20** as a chromatographically pure solid: ^{1}H NMR (DMSO-d_6) δ 0.82 (t, 3H, CH_3-9), 0.87 (d, 3H, CH_3-1), 1.01 (m, 2H, CH_2-8), 1.20 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 1.81 (m, 2H, CH_2-4), 3.77 (m, 1H, CH-3), 4.01 (m, 1H, CH-2), 8.49 (s, 2H, H-2 and H-5). Anal. ($C_{11}H_{21}N_3O$) C, H, N.

1-(2-Keto-3-nonyl)-1,2,3,4-tetrazole (22) and 2-(2-Keto-3-nonyl)-1,2,3,4-tetrazole (24). The title compounds were prepared according to the general procedure I. Purification by silica gel column flash chromatography eluting with c-C_6H_{12}–$CHCl_3$ (70:30) gave 46% of the faster moving **24**. Elution was then carried on with c-C_6H_{12}–$CHCl_3$ (50:50) to give 28% yield of **22**: ^{1}H NMR (DMSO-d_6) δ 0.82 (t, 3H, CH_3-9), 0.97 (m, 2H, CH_2-8), 1.20 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 2.13 (s, 3H, CH_3-1), 2.05 and 2.27 (m, 2H, CH_2-4), 5.76 (m, 1H, CH-3), 9.48 (s, 1H, H-5). Anal. ($C_{10}H_{19}N_4O$) C, H, N.

24: ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, CH_3-9) 0.94 (m, 2H, CH_2-8), 1.19 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 2.12 (s, 3H, CH_3-1), 2.24 (m, 2H, CH_2-4), 5.98 (m, 1H, CH-3), 9.05 (s, 1H, H-5). Anal. ($C_{10}H_{19}N_4O$) C, H, N.

1-(2-Hydroxy-3-nonyl)-1,2,3,4-tetrazole (23). The title compound was prepared according to the general procedure II. Purification by silica gel column flash chromatography eluting with $CHCl_3$–c-C_6H_{12} (80:20) gave 85% yield of **23** as a mixture of two diastereoisomers which were separated by preparative TLC plate eluting with $CHCl_3$–c-C_6H_{12} (80:20).

erythro-**23e**: ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, CH_3-9), 0.92 (d, 3H, J = 6.4 Hz, CH_3-1), 1.20 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.98 (m, 2H, CH_2-4), 3.93 (m, 1H, CH-3), 4.55 (m, 1H, CH-2), 9.43 (s, 1H, H-5). Anal. ($C_{10}H_{20}N_4O$) C, H, N.

threo-**23t**: ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H, CH_3-9), 0.96 (d, 3H, J = 6.3 Hz, CH_3-1), 1.17 (m, 8H, CH_2-5, CH_2-6, CH_2-7, CH_2-8), 1.89 (m, 2H, CH_2-4), 3.91 (m, 1H, CH-3), 4.49 (m, 1H, CH-2), 9.38 (s, 1H, H-5). Anal. ($C_{16}H_{20}N_4O$) C, H, N.

2-(2-Hydroxy-3-nonyl)-1,2,3,4-tetrazole (25). The title compound was prepared according to the general procedure II. Purification by silica gel column flash chromatography eluting with c-C_6H_{12}–$CHCl_3$ (60:40) gave 74% yield of **25** as a mixture of two diastereoisomers which were separated by preparative HPLC eluting with c-C_6H_{12}–AcOEt (70:30) (2.4 mL/min).

erythro-**25e**: ^{1}H NMR (DMSO-d_6) δ 0.84 (m, 6H, CH_3-1 and CH_3-9), 1.18 (m, 6H, CH_2-5, CH_2-6, CH_2-7), 2.05 (m, 2H, CH_2-4), 3.96 (m, 1H, CH-3), 4.72 (m, 1H, CH-2), 9.00 (s, 1H, H-5). Anal. ($C_{10}H_{20}N_4O$) C, H, N.

threo-**25t**: ^{1}H NMR (DMSO-d_6) δ 0.82 (m, 3H, CH_3-9), 1.19 (m, 9H, CH_2-5, CH_2-6, CH_2-7, CH_3-1), 1.92 (m, 2H, CH_2-4), 4.00 (m, 1H, CH-3), 4.72 (m, 1H, CH-2), 8.96 (s, 1H, H-5). Anal. ($C_{10}H_{20}N_4O$) C, H, N.

Biological Studies. Enzyme Assay. The method used for the determination of activity against adenosine deaminase has been described in a preceding paper.[14]

Acknowledgment. This work was supported by the Italian Research Council (CNR, Roma) Progetto finalizzato *Chimica Fine* and by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST). We thank M. Brandi, F. Lupidi and G. Rafaiani for technical assistance.

References

(1) Van Der Weyden, M. B.; Kelly, W. N. *Adenosine* deaminase deficiency and severe combined immunodeficiency disease. *Life Sci.* 1977, *20*, 1645–1650.
(2) Thompson, L. F.; Seegmiller, J. E. *Adenosine* deaminase deficiency and severe combined immunodeficiency disease. *Adv. Enzymol.* 1980, *54*, 167–210.
(3) Murray, J. L.; Loftin K. C.; Munn, C. G.; Reuben, J. M.; Mansell, P. W. A.; Hersh, E. M. Elevated adenosine deaminase and purine nucleoside phosphorilase activity in peripheral blood null lymphocytes from patients with aquired immune deficiency syndrome. *Blood* 1985, *65*, 1318–1324.
(4) Wilson, D. K.; Rudolph, F. B.; Quiocho, F. A. Atomic structure of adenosine deaminase with transition-state analogs: understanding catalysis and immunodeficiency mutations. *Science* 1991, *252*, 1278–1284.
(5) Glazer, R. I. Adenosine deaminase inhibitors: their role in chemotherapy and immunosuppression. *Cancer Chemother. Pharmacol.* 1980, *4*, 227–235.
(6) Glazer, R. I. 2'-Deoxycoformycin and other adenosine deaminase inhibitors. *Rev. Drug Metab. Drug Interact.* 1980, *3*, 105–128.
(7) Lee, N.; Russell, N. H.; Ganeshaguru, K.; Jackson, B. F. A.; Piga, A.; Prentice, H. G.; Foa, R.; Hoffbrand, V. Mechanism of deoxyadenosine toxicity in human lymphoid cells in vitro: relevance to the therapeutic use of inhibitors of adenosine deaminase. *Br. J. Haemat.* 1984, *56*, 107–119.
(8) McIlwain, H. In *Central nervous system studies on metabolic regulation and function*; Genazzari, E., Herken, H., Eds.; Springer-Verlag: New York, 1983; pp 3–11.
(9) Stone, T. W. Purine receptors and their pharmacological roles. *Adv. Drug Res.* 1989, *18*, 291–429.
(10) Agarwal, R. P. Inhibitors of adenosine deaminase. *Pharmacol. Ther.* 1982, *17*, 399–429.
(11) Phillis, J. W.; De Long, E. R.; Towner, K. J. Adenosine deaminase inhibitors enhance cerebral anoxic hyperemia in the rat. *J. Cereb. Blood Flow Metab.* 1985, *5*, 295–299.
(12) Centelles, J. J.; Franco, R.; Bozal, J. Purification and partial characterization of brain adenosine deaminase: inhibition by purine compounds and by drugs. *J. Neurosci. Res.* 1988, *19*, 258–267.
(13) North, T. W.; Cohen, S. S. Aranucleosides and aranucleotides in viral chemotherapy. *Pharmacol. Ther.* 1979, *4*, 81–108.
(14) Antonini, I.; Cristalli, G.; Franchetti, P.; Grifantini, M.; Martelli, S.; Lupidi, G.; Riva, F. Adenosine deaminase inhibitors. Synthesis of deaza analogues of *erythro*-1-(2-hydroxy-3-nonyl)adenine. *J. Med. Chem.* 1984, *27*, 274–278.
(15) Lupidi, G.; Cristalli, G.; Marmocchi, F.; Riva, F.; Grifantini, M. Inhibition of adenosine deaminase from several sources by deaza derivatives of adenosine and EHNA, *J. Enzyme Inhib.* 1985, *1*, 67–75.
(16) Cristalli, G.; Franchetti, P.; Grifantini, M.; Vittori, S.; Lupidi, G.; Riva, F.; Bordoni, T.; Geroni, C.; Verini, M. A. Adenosine deaminase inhibitors. Synthesis and biological activity of deaza analogues of *erythro*-1-(2-hydroxy-3-nonyl)adenine. *J. Med. Chem.* 1988, *31*, 390–393.
(17) Cristalli, G.; Eleuteri, A.; Franchetti, P.; Grifantini, M.; Vittori, S.; Lupidi, G. Adenosine deaminase inhibitors. Synthesis and structure-activity relationships of imidazole analogues of *erythro*-1-(2-hydroxy-3-nonyl)adenine. *J. Med. Chem.* 1991, *34*, 1187–1192.
(18) 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 analogs. *Drug Dev. Res.* 1993, *28* (3), 253–258.
(19) Bongini, A.; Cainelli, F.; Contento, M.; Manescalchi, F. Anion-exchange resins as halogen carriers in the bromination and chlorobromination of organic compounds. *Synthesis* 1980, *232*, 143–145.
(20) Takeuchi, Y.; Yeh, H. J. C.; Kirk, K. L.; Cohen, L. A. *J. Org. Chem.* 1978, *43*, 3565–3570.
(21) Schaeffer, H. J.; Schwender, C. F. Enzyme inhibitors. 26. Bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl)adenines. *J. Med. Chem.* 1974, *17*, 6–8.