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ADENOSINE DEAMINASE INHIBITORS. SYNTHESIS AND BIOLOGICAL EVALUATION OF CHAIN MODIFIED ANALOGS OF (+)-EHNA.

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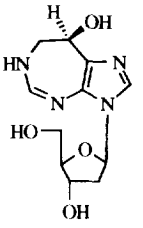
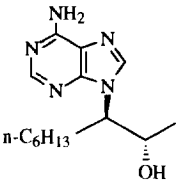
Abstract: Unsaturated chain modified analogs of (+)-EHNA have been prepared and evaluated as inhibitors of adenosine deaminase. The geometry appears to play a role in binding to the enzyme.

Adenosine deaminase (ADA, adenosine aminohydrolase, E.C.3.5.4.4) is an enzyme in the purine catabolic pathway responsible for the conversion of adenosine and 2'-deoxy adenosine to inosine and 2'-deoxyinosine, respectively.[1] ADA deficiency is associated with lymphotoxic elevations in the concentrations of 2'-deoxyadenosine and dATP, which result in a severe combined immunodeficiency syndrome (SCIDS).[2] This association of ADA deficiency with SCIDS has created an interest in the pharmacologic inhibition of ADA as potential cytotoxic therapy of malignant lymphoproliferative diseases.[3] Another benefit of ADA inhibition is to prevent deamination and subsequent deactivation of important chemotherapeutic agents that contain adenine bases such as arabinofuranosyladenine (ara-A), 8-azaadenosine, formycin A, and 2',3'-dideoxy-adenosine.[4] An additional benefit of ADA inhibition is protection of injured tissues in cerebral and myocardial ischemia. This is due to increased levels of adenosine, which appear to limit the extent of degeneration.[5] Two important ADA inhibitors are 2'-deoxycoformycin **1** (pentostatin) and *erythro*-9-(2-hydroxy-3-nonyl)-adenine (EHNA)**2**.[6]



1, dCF



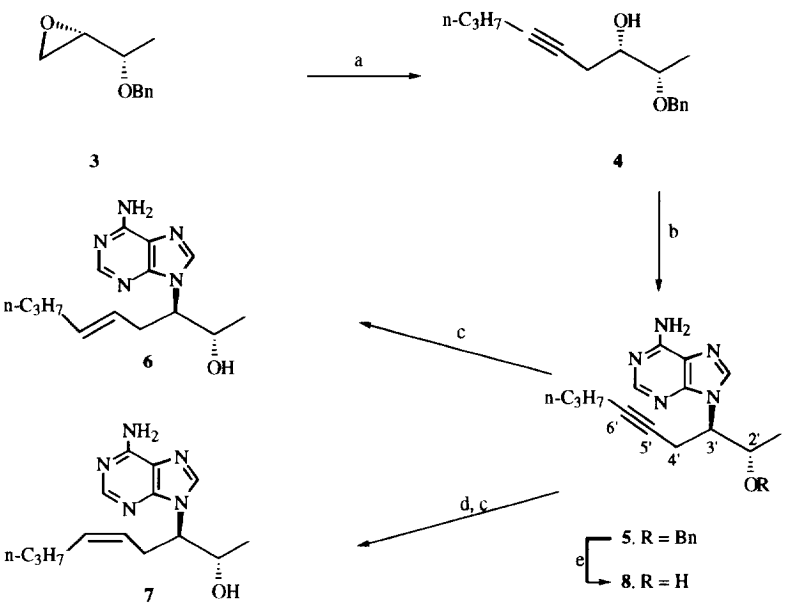
2, (+)-EHNA

Structure-activity relationship (SAR) studies of analogs of EHNA are an ongoing program in our laboratory. We have recently reported the synthesis of C-1' and nor C-1' derivatives of (+)-EHNA and 3-deaza-(+)-EHNA[7] as well as chain hydroxylated analogs believed to be putative metabolites in (+)-EHNA metabolism.[8] These hydroxylated analogs were less active than EHNA confirming the need for hydrophobicity at the chain terminus.

Although ADA interaction with a tight binding transition state inhibitor has been obtained by X-ray analysis,[9] no comparable data for a semi-tight binding inhibitor such as EHNA are available. Neither the conformation of the chain nor its binding site have been elucidated. To better assess the role of the alkyl chain in ADA inhibition, we decided to study the effects of conformation on activity. The introduction of some rigidity in the alkyl chain at the 5',6'-position was thus undertaken. The choice of this position was simply based on synthetic expediency where the four carbon fragment, derived from *L*-ascorbic acid, is added to a five carbon chain with varied functionalities, as we previously reported.[8]

The reaction of epoxide **3**[10] with 1-pentynyl lithium gave alcohol **4**. Construction of the purine ring has been previously accomplished by a rather lengthy procedure through the intermediacy of an amine.[8] A more recent approach to 9-substituted purine rings made use of the Mitsunobu reaction between an already formed purine and an alcohol.[11] Application of this strategy to condense **4** with adenine was unsuccessful and resulted, instead, in forming what is believed to be olefinic products as suggested by TLC analysis. On the other hand, condensation of **4** with 6-chloropurine furnished a product that could not be obtained in pure form due to its similar chromatographic behavior to dihydro-diisopropylazodicarboxylate, a by-product in the Mitsunobu reaction. However, ^{1}H NMR analysis of the crude product clearly showed the formation of a chloropurine derivative as evidenced by two singlets at δ 8.0 and 8.33 corresponding to H-2 and H-8, respectively. Ammonolysis of this product allowed the isolation of a compound that proved to be an 85:15 mixture of N-9:N-7 isomers. Separation of these isomers was accomplished by preparative thin-layer chromatography, which furnished the pure N-9 isomer **5** whose structural assignment was derived from UV analysis.

Intermediate **5** was successfully converted to three different derivatives **6**, **7**, and **8**. The synthetic routes are depicted in the following scheme. E-isomer **6** was obtained by treatment with sodium/ammonia, which resulted in the simultaneous reduction of the triple bond and cleavage of the benzyl ether. On the other hand, partial catalytic hydrogenation over Lindlar's catalyst followed by sodium/ ammonia reductive cleavage of the benzyl group gave the Z-isomer **7**. Finally, the acetylenic compound **8** was prepared by selective debenzylation of **5** using calcium/ammonia with concommitent formation of minor amounts of **6**.[12]



(a) n-BuLi, 1-pentyne, Li_2CuCl_4, THF, -30 °C, 88%; (b) i. 6-Chloropurine, PPh_3, DIAD, THF, reflux, 24 h. ii. NH_3, 90 °C, 27% (two steps); (c) Na/NH_3, toluene, -78 °C, 80%; (d) Lindlar's catalyst, toluene, H_2, 99%; (e) Ca/NH_3, toluene, -78 °C, 30% (**8**), 20% (**6**).

The inhibitory activity of compounds **2**, **6**, **7**, and **8** on calf intestinal mucosa ADA was evaluated in a single experiment following published procedures.[8] The findings are summarized below:

Compound	K_i (nM)
2	1.13
7	3.74
6	50.70
8	188.00

As can be seen, all three compounds have lower activity than (+)-EHNA, isomer **7** being the most active in this new series and is three-fold less active than EHNA itself. It is thirteen and fifty times more active than **6** and **8**, respectively. This can be attributed to distance-related hydrophobic interactions between the terminus and the purine ring where they are optimal in the **Z**-isomer **7**.

Additional efforts are in progress to further support this hypothesis by evaluating analogs that can better interact with the heterocycle.

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12. The 1H NMR data (300 MHz, CDCl3) for compounds **6**, **7**, and **8** follow: **6**: 0.74 (t, J = 7.2 Hz, 3H), 1.14-1.25 (m, 2H), 1.78-1.85 (m, 2H), 2.59-2.80 (m, 2H), 4.22 (ddd; J = 10.5, 3.9, 2.1 Hz, 1H), 4.33 (dq, J = 6.3, 2.0, 1H), 5.10-5.30 (m, 2H), 5.60 (bs, 1H, D2O exchangeable), 5.88 (bs, 2H, D_2O exchangeable), 7.70 (s, 1H), 8.3 (s, 1H); **7**: 0.70 (t, J = 7.4 Hz, 3H), 0.9-1.2 (m, 2H), 1.36 (d, J = 6.5 Hz, 3H), 1.65 (ddt, J = 4.0, 15.0, 7.5 Hz, 2H), 2.55-2.65 (m, 1H), 2.85-3.00 (m, 1H), 4.17 (dq, J = 12.0, 3.5, 3.0 Hz, 1H), 4.35 (d of ABq, J = 12.0, 6.0, 1.5 Hz, 1 H), 5.15-5.25 (m, 1H), 5.35-5.45 (m, 1H), 5.82 (bs, 2H, D_2O exchangeable), 7.7 (s, 1H), 8.32 (s, 1H); **8**: 0.86 (t, J = 7.0 Hz, 3H), 1.34 (d, J = 6.6 Hz, 3H), 1.41 (Sextet, J = 7.3, 7.24, 7.2 Hz, 2H), 2.0 (tt, J = 7.0, 2.4, 2.2 Hz, 2H), 2.7 (dd, J =5.6, 2.4 Hz, 1H), 2.75 (dd, J = 5.5, 2.4 Hz, 1H), 4.3 (dq, J = 2.4 Hz, 1H), 4.4 (dq, J = 13.8, 7.3, 1.7, 1H), 5.85 (bs, 1H, D_2O exchangeable), 6.05 (bs, 2H, D_2O exchangeable). 7.9 (s, 1H), 8.3 (s, 1H).

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