(R)-(-)- and (S)-(+)-Adenallene: Synthesis, Absolute Configuration, Enantioselectivity of Antiretroviral Effect, and Enzymic Deamination

Sreenivasulu Megati,† Zafrir Goren,† James V. Silverton,‡ Joel Orlina,‡ Hisao Nishimura,§ Takuma Shirasaki,‖ Hiroaki Mitsuya,‖ and Jiri Zemlicka*,†,⊥

Department of Chemistry, Michigan Cancer Foundation, Detroit, Michigan 48201, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892, Chiral Technologies, Inc., Exton, Pennsylvania 19341, The Clinical Oncology Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, and Departments of Internal Medicine and Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201

Received March 12, 1992

Synthesis of optically pure (-)- and (+)-adenallene **2** and **3** is described. Racemic adenallene (**1a**) was subjected to deamination with adenosine deaminase monitored by HPLC using a Chiralcel CA-1 column to give (-)-adenallene (**2**) and (+)-hypoxallene (**4**). The latter compound was converted to acetate **5**. The reaction of **5** with trifluoromethanesulfonic anhydride and pyridine followed by ammonolysis furnished acetate **6** or (+)-adenallene (**3**) depending on the solvent used in the last step. Acetate **5** was smoothly transformed to the 6-chloro derivative **7**, but an attempted ammonolysis led only to racemization and decomposition. Single crystal X-ray diffraction established the *R*-configuration of (-)-enantiomer **2**. The latter forms a pseudosymmetric dimer in the lattice with the adenine moiety in an anti-like conformation. The torsional angles of the allenic bonds show departures from 90° (91 and 97°, respectively) and rotameric preference of the hydroxymethyl groups is different in both molecules of the dimer. The *R*-enantiomer **2** inhibited the replication and cytopathic effect of human immunodeficiency virus (HIV-1) in ATH8 cell culture with an IC_{50} of 5.8 μM, whereas the *S*-enantiomer **3** was less active (IC_{50} > 200 μM). The enantioselectivity of the anti-HIV effect is significantly lower than that of 2′,3′-dideoxyadenosine. Kinetics of deamination of *R*- and *S*-enantiomers **2** and **3** catalyzed by adenosine deaminase gave the following parameters: K_m values of *R*-form **3** and *S*-form **2** were 0.41 and 0.52 mM with V_{max} being 530 and 18.5 μmol/min, respectively. Again, a much lower level of enantioselectivity of deamination was observed than that of D- and L-adenosine. These results indicate (i) different enantioselectivity of enantiomers **2** and **3** as HIV inhibitors and adenosine deaminase substrates and (ii) both *R*- and *S*-enantiomers **2** and **3** can function as nucleoside analogues with varied enantioselectivity for different enzymes or receptors.

Recent work in our laboratory led to a synthesis of a novel class of nucleoside analogues, allenols of the type **1** derived from nucleic acid bases.[1-6] Modified procedures for preparation of adenallene (**1a**) and cytallene (**1b**) were also reported.[7-9] (±)-Adenallene (**1a**) and -cytallene (**1b**) were found[10-12] to efficiently inhibit in vitro replication and cytopathic effect of human immunodeficiency viruses HIV-1 and HIV-2, etiologic agents of acquired immunodeficiency syndrome (AIDS). However, investigations of **1a** and **1b** in Friend or Rauscher leukemia virus cultures,[6] have shown a lower antiretroviral potency. These differential effects may depend on a degree of phosphorylation of **1a** and **1b** in the target cells. Allenols **1a** and **1b** are considered analogues of the corresponding nucleosides, 2′,3′-dideoxyadenosine (ddAdo) and -cytidine (ddCyd), which are also active antiretroviral agents, on the basis of biological activity[10,11] and molecular models.[1,2] It was also shown that (±)-cytallene (**1b**) but not (±)-adenallene (**1a**)

† Michigan Cancer Foundation.
‡ National Heart, Lung, and Blood Institute.
§ Chiral Technologies, Inc.
‖ National Cancer Institute.
⊥ Wayne State University School of Medicine.

(1) Phadtare, S.; Zemlicka, J. Allenic Derivatives of Nucleic Acid Components and Their Transformation Products. *Nucleic Acids Symp. Ser.* **1987**, *18*, 25-28.
(2) Phadtare, S.; Zemlicka, J. Nucleic Acids Derived Allenols: Unusual Analogues of Nucleosides with Antiretroviral Activity. *J. Am. Chem. Soc.* **1989**, *111*, 5925-5931.
(3) Phadtare, S.; Zemlicka, J. Allenic Derivatives of Nucleic Acid Bases—New Acyclic-Nucleoside Analogues with Antiretroviral Activity. *Nucleic Acids Symp. Ser.* **1988**, *20*, 39-40.
(4) Phadtare, S.; Zemlicka, J. Synthesis of N^1-(4-Hydroxy-1,2-butadien-1-yl)thymine, an Analogue of 3′-Deoxythymidine. *J. Org. Chem.* **1989**, *54*, 3675-3679.
(5) Phadtare, S.; Kessel, D.; Zemlicka, J. Unsaturated Nucleoside Analogues: Synthesis and Antitumor Activity. *Nucleosides Nucleotides* **1989**, *8*, 907-910.
(6) Phadtare, S.; Kessel, D.; Corbett, T. H.; Renis, H. E.; Court, B. A.; Zemlicka, J. Unsaturated and Carbocyclic Nucleoside Analogues: Synthesis, Antitumor, an Antiviral Activity. *J. Med. Chem.* **1991**, *34*, 421-429.
(7) Khorlin, A. A.; Smirnov, I. P.; Kochetkova, S. V.; Tsilevich, T. L.; Shchaveleva, I. L.; Gottikh, B. P.; Florent'ev, V. L. Compounds Similar to Acyclovir. IV. Convenient Method of Synthesizing Adenallene. *Bioorg. Khim.* **1989**, *15*, 530-533 (English translation **1990**, *15*, 291-293).
(8) Kochetkova, M. V.; Tsytovich, A. V.; Mitsner, B. I. A Convenient Approach to the Synthesis of Nucleic Acid Bases Allenic Derivatives Possessing Anti-HIV Activity. *Nucleic Acids Symp. Ser.* **1991**, *24*, 233.
(9) Tsytovich, A. V.; Kochetkova, M. V.; Kuznetsova, E. V.; Mitsner, B. I.; Shvets, V. I. Acyclic Nucleoside Analogues. I. Development of Allenic Nucleoside Derivatives Synthesis. *Bioorg. Khim.* **1991**, *17*, 1086-1093 (in Russian).
(10) Hayashi, S.; Phadtare, S.; Zemlicka, J.; Matsukura, M.; Mitsuya, H.; Broder, S. Adenallene and Cytallene: Acyclic Nucleoside Analogues that Inhibit Replication and Cytopathic Effect of Human Immunodeficiency Virus. *In Vitro. Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6127-6131.
(11) Hayashi, S.; Phadtare, S.; Zemlicka, J.; Matsukura, M.; Mitsuya, H.; Broder, S. Adenallene and Cytallene, Two Novel Acyclic Nucleoside Derivatives Active against Human Immunodeficiency Virus (HIV) in T-Cells and Monocytes/Macrophages in Vitro: Further Characterization of Anti-Viral and Cytotoxic Activity. Mechanisms of Action and Therapeutic Applications of Biologicals in Cancer and Immune Deficiency Disorders; Alan R. Liss, Inc.: New York, 1989; pp 371-383.
(12) Larder, B. A.; Chesebro, B.; Richman, D. D. Susceptibilities of Zidovudine-Susceptible and -Resistant Human Immunodeficiency Virus Isolates to Antiviral Agents Determined by Using a Quantitative Plaque Reduction Assay. *Antimicrob. Agents Chemother.* **1990**, *34*, 436-441.

0022-2623/92/1835-4098$03.00/0

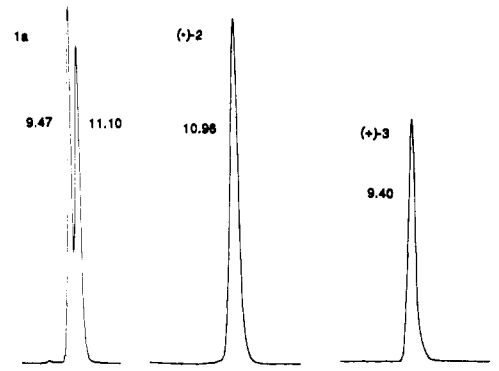


Figure 1. Resolution of (±)-adenallene (**1a**) on Chiralcel CA-1 column; elution with ethanol, flow rate 0.5 mL/min, column temperature 40 °C. Retention time (t_R, min) is indicated at each peak.

was phosphorylated by appropriate 2′-deoxynucleoside kinase.[13-15] Unlike 2′,3′-dideoxyribonucleosides which are D-enantiomers, allenols **1a** and **1b** have not been obtained to date in optically pure form. The previous results have indicated[10] that both (±)-adenallene (**1a**) and -cytallene (**1b**) exhibit an anti-HIV effect approximately equal to that of both 2′,3′-dideoxyribonucleoside counterparts. It seems then likely that one of the enantiomers (*R* or *S*) will be more potent than the racemic mixture **1a** or **1b**. In addition, elimination of an unwanted (inactive) enantiomer may also reduce toxicity and side effects in therapeutical applications.

Synthesis of the optically pure biologically active allenols derived from nucleic bases is therefore of utmost interest. In this contribution, we describe resolution of (±)-adenallene (**1a**), synthesis of optically pure enantiomers **2** and **3**, their absolute configuration, anti-HIV activity, and kinetics of deamination catalyzed with adenosine deaminase (ADA).

Synthesis

The HPLC experiments have indicated that (±)-adenallene[2] (**1a**) and 2-aminoadenallene (**1c**) can be resolved on a Chiralcel CA-1 column (silica gel coated with cellulose triacetate as a chiral stationary phase) using ethanol as an eluent at 40 °C (Figure 1). Attempted resolution of hypoxallene (**1d**), cytallene (**1b**), and methylcytallene (**1e**) was not successful. Although small samples of the required enantiomers of (±)-adenallene (**1a**) could probably be obtained by this method, any larger scale resolution would be prohibitively expensive. Attempted low-pressure chromatography of **1a** on a column of cellulose triacetate[16] using water as an eluent led to some separation of enantiomers (data not shown) but repeated chromatography would be necessary to obtain optically pure enantiomers.

Our previous results[2] have indicated that deamination of (±)-adenallene (**1a**) catalyzed by ADA could become a basis for kinetic resolution of both *R*- and *S*-enantiomers. Indeed, deamination of **1a** with ADA from calf intestine,

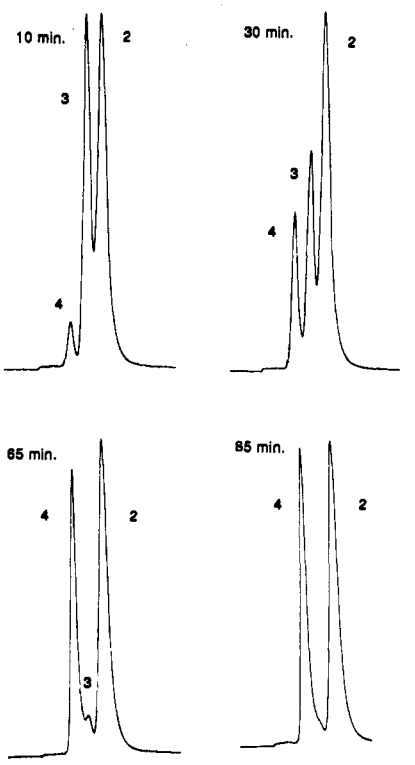


Figure 2. Time course of deamination of (±)-adenallene (**1a**) with adenosine deaminase (ADA). Chromatography was performed as described in Figure 1. For other details, see Experimental Section. t_R 7.56–7.61 (compound **4**), t_R 9.28–9.42 (compound **3**), t_R 11.07–11.16 (compound **2**).

conveniently monitored by HPLC on a Chiralcel CA-1 (Figure 2), gave (−)-adenallene (**2**) in 78% yield. The yield of the deaminated product, (+)-hypoxallene (**4**), was 90%. Both compounds were obtained optically pure after a single recrystallization. Thus, (−)-enantiomer **2** gave a single peak on Chiralcel CA-1 column (Figure 1). (+)-Hypoxallene (**4**) was converted to acetate **5** (82%, Scheme I). The reaction of **5** with trifluoromethanesulfonic anhydride in the presence of pyridine and subsequent ammonolysis in dioxane[17] gave (+)-adenallene acetate **6** in 22% yield. Deacetylation of **6** gave optically pure (+)-adenallene (**3**, Figure 1) in 90% yield. The first step involving trifluoromethanesulfonic anhydride and pyridine appears to be the limiting factor in the conversion of **5** to **3** because a direct ammonolysis with ammonia in methanol gave **3** in 20% yield. Several attempts to improve the yield of **3** by using alternate literature procedures[18-20] for activation of the purine 6 position were fruitless. Chlorination[21,22] of **5** was successful (81% yield of **7**), but the subsequent

(13) Eriksson, S.; Kierdaszuk, B.; Munch-Petersen, H.; Öberg, B.; Johansson, N. G. Comparison of the Substrate Specificities of Human Thymidine Kinase 1 and 2 and Deoxycytidine Kinase Toward Antiviral and Cytostatic Nucleoside Analogs. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 586–592.

(14) Kierdaszuk, B.; Bohman, C.; Ullman, B.; Eriksson, S. Substrate Specificity of Human Deoxycytidine Kinase Toward Antiviral 2′,3′-Dideoxynucleoside Analogs. *Biochem. Pharmacol.* **1992**, *43*, 197–206.

(15) Kessel, D., unpublished results.

(16) Hesse, G.; Hagel, R. Chromatographic Resolution of Racemates. *Liebigs Ann. Chem.* **1976**, 996–1008.

(17) Herdewijn, P.; Van Aerschot, A. Synthesis of 9-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-2,6-diaminopurine (AzddDAP). *Tetrahedron Lett.* **1989**, *30*, 855–858.

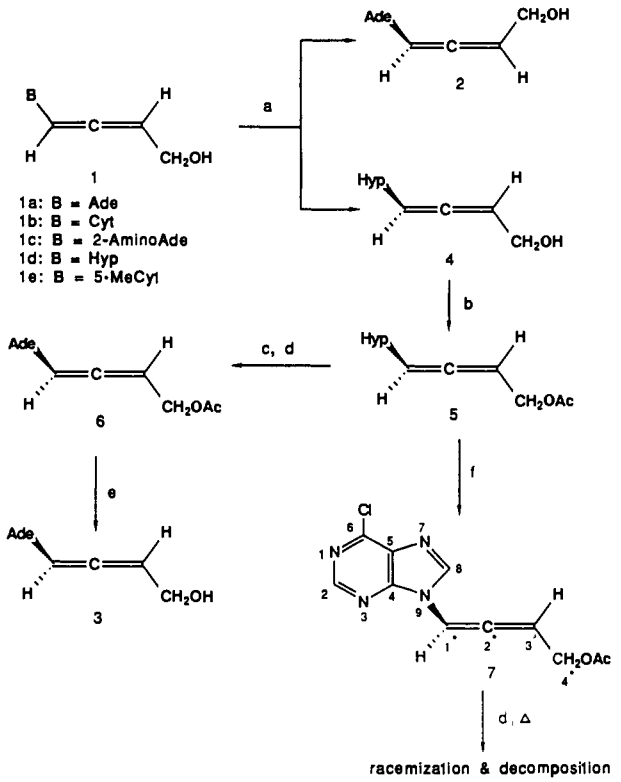
(18) Reese, C. B.; Skone, P. A. The Protection of Thymine and Guanine Residues in Oligonucleotide Synthesis. *J. Chem. Soc. Perkin Trans. I* **1984**, 1263–1271.

(19) Adamiak, R. V.; Biala, E.; Skalski, B. New, Ionic Side-Products in Oligonucleotide Synthesis: Formation and Reactivity of Fluorescent N-/Purin-6-yl/pyridinium Salts. *Nucleic Acids Res.* **1985**, *13*, 2989–3003. Dimethylamine instead of ammonia was erroneously given in the procedure for 2′,3′,5′-tri-*O*-acetyladenosine.

(20) Fathi, R.; Goswami, B.; Kung, P.-P.; Gaffney, B. L.; Jones, R. A. Synthesis of 6-Substituted 2′-Deoxyguanosine Derivatives Using Trifluoroacetic Anhydride in Pyridine. *Tetrahedron Lett.* **1990**, *31*, 319–322.

(21) Zemlicka, J.; Sorm, F. Nucleic Acids Components and Their Analogues. LX. The Reaction of Dimethylchloromethyleneammonium Chloride with 2′,3′,5′-Tri-O-acetylinosine. A New Synthesis of 6-Chloro-9-(β-D-ribofuranosyl)purine. *Collect. Czech. Chem. Commun.* **1965**, *30*, 1880–1889.

Scheme I [a]



[a] (a) ADA, pH 7.5; (b) Ac_2O, pyridine; (c) $(CF_3SO)_2O$, pyridine, CH_2Cl_2; (d) NH_3, dioxane; (e) NH_3, MeOH; (f) $[(CH_3)_2N{=}CHCl]^+Cl^-$, $CHCl_3$. For abbreviations of nucleic acids components used in Scheme I and in the text see ref 51. Thus, Ade = adenin-N^9-yl, Hyp = hypoxanthin-N^9-yl, Gua = guanin-N^9-yl, Cyt = cytosin-N^1-yl.

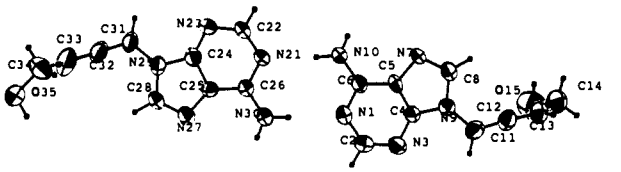


Figure 3. ORTEP drawing of the two independent molecules as found in the crystal of (*R*)-(−)-adenallene (2). Note the nearly symmetrical relationship except for the hydroxymethyl group.

ammonolysis (heating in a sealed tube at 100 °C for several hours) led only to decomposition (formation of adenine) and racemization of product 3. It is to be noted that the aforementioned method constitutes the first synthesis of optically pure enantiomers of a heterocyclic allene.

Crystallographic Results

A. Absolute Configuration and Packing. The nature of the crystal packing is relevant to later discussion and will be described first. The absence of large intensities for *h*0*l* reflections with *l* odd is explained by the pseudosymmetrical relationship between the two independent molecules of (−)-adenallene (2) in the asymmetric unit shown by an ORTEP drawing[23] (Figure 3). The configuration of 2 as defined by Cahn–Ingold–Prelog notation[24] is *R*. Figure 4 is an ORTEP drawing[23] depicting the

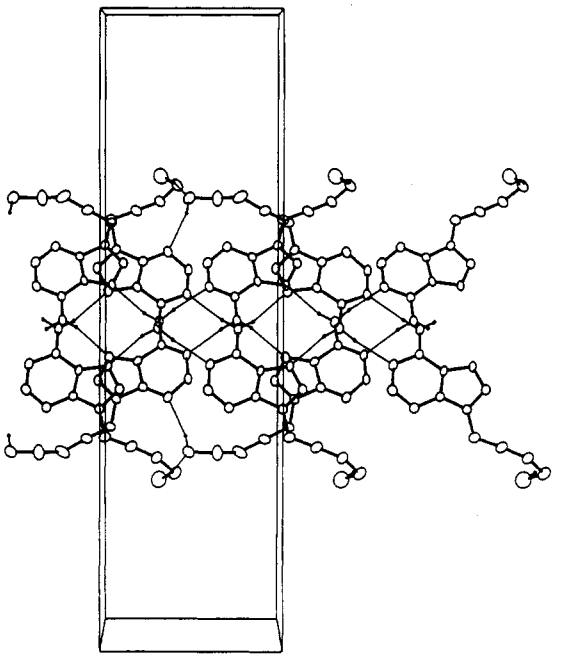


Figure 4. Packing in the crystal of (*R*)-(−)-adenallene showing hydrogen bonding. While a conventional unit cell is delineated, only the hydrogen-bonded bilayer is shown. The drawing was produced by ORTEP and the direction of projection is c.

Table I. Hydrogen Bonds (distances in Å and angles in degrees)

donor (D)	H	acceptor (A)	A···H	D···A	D–H–A angle
N1	H13	N30	2.15	3.065 (4)	173
N27	H15	N10	2.20	2.970 (4)	165
N21	H16	N10	2.11	3.012 (4)	173
N7	H14	N30	2.12	2.965 (4)	166
O35	H36	O15	1.81	2.714 (6)	163
N23	H35	O35	2.03	2.811 (4)	139

hydrogen bonding and packing. The basic motif is a hydrogen bonded bilayer with the individual essentially planar component layers linked by relatively weak N–H···N hydrogen bonds and also by O–H···N bonds (Table I). The parallel layers are joined by fairly short O–H···O hydrogen bonds. Notable is the absence of Hoogsteen base-pairing[25] in the dimer. Instead, the N···N hydrogen bonds linking only pyrimidine residues of adenine resemble those once considered for a double helical structure of poly-8-bromoadenylic acid.[26,27] The primitive cell comprises one repeating unit of the bilayer. The oxygen atoms of the independent molecules form hydrogen bonds in different fashions: one type reinforcing the infinite layer and the other joining the two layers.

B. Molecular Conformation. The crystal conformations and relative positions of the two independent molecules are shown in Figure 3. The molecular dimensions (see the supplementary material) are close to literature values. The figure also shows the pseudosymmetry with the only major deviation from a 2-fold axis being in the position of the hydroxymethyl groups. The molecules show only small deviations from coplanarity.

(22) Zemlicka, J.; Owens, J. 6-Chloro-9-β-D-ribofuranosylpurine, a Versatile Intermediate in the Synthesis of Purine Ribonucleosides. In *Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods, and Techniques*; Townsend, L., B., Tipson, R. S., Eds.; John Wiley & Sons: New York, 1978; Part 2, pp 611–614.
(23) Johnson, C. K. ORTEP, Technical Report, OR-3794, Oak Ridge National Laboratory, TN, 1965.
(24) Cahn, R. S.; Ingold, C.; Prelog, V. Specification of Molecular Chirality. *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 385–415.
(25) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 122–126.
(26) Howard, F. B.; Frazier, J.; Miles, H. T. Poly-8-bromoadenylic Acid. A Helical, All Syn Homopolynucleotide. *J. Biol. Chem.* **1974**, *249*, 2987–2990.
(27) Govil, G.; Fisk, C.; Howard, F. B.; Miles, H. T. Structure of Poly 8-Bromoadenylic Acid: Conformational Studies by CPF Energy Calculations. *Nucleic Acids Res.* **1977**, *4*, 2573–2592.

Table II. Conformationally Significant Torsion Angles (in degrees)

atom 1	atom 2	atom 3	atom 4	angle
C34	C33	C31	N29	−96.8 (4)
C14	C13	C11	N9	−87.8 (5)
O35	C34	C33	C32	146.0 (4)
O15	C14	C13	C12	−8.6 (7)
C14	H4	N9	H3	90.9
C34	H9	N29	H10	82.1
C24	N29	C31	C32	−161.7 (4)
C28	N29	C31	C32	11.0 (6)
C4	N9	C11	C12	−177.2 (3)
C8	N9	C11	C12	5.3 (6)

The oxygen atom positions appear to be constrained by the hydrogen bonds and the atoms have quite different roles in the packing. In molecule 1, the O15–C14–C13–C12 torsion angle is −8.6°, i.e. eclipsed by the allene group and, in molecule 2, the C35–C34–C33–C32 angle is 146.0° (Table II). A rough calculation using XICAMM[28] indicates that torsion angles of −20–20° and ±(110–150°) are the most likely energetically and thus the observed values lie in different but likely zones. The torsion angles in Table II indicate that neither molecule has exactly a 90° angle of allenic bonds associated with sp-hybridized C12 and C32.

Our previous molecular modeling studies showed a good correspondence between (S)-adenallene (**3**) and the 3′-endo conformer of adenosine.[2] More recently, stereochemical rationalizations of compounds showing promise in AIDS therapy including allenols **1a** and **1b** were discussed.[29,30] It was stated that adenallene (presumably the S-enantiomer) mimics the 3′-exo conformation of the furanose ring and ap rotamer of the hydroxymethyl group in nucleosides.[30] A global energy minimum was determined by molecular mechanics for (S)-cytallene.[29] Unfortunately, no numerical values were given and one cannot assess whether the depicted global energy minimum is much different from other local minima. In 2′,3′-dideoxyribonucleosides it is possible that the ap conformation usually adopted by the hydroxymethyl group may represent a significant characteristic of antiretroviral drugs but this does not seem relevant[31] to the allenols of the type 1. The energy barriers among the preferred conformations of the hydroxymethyl groups appear to be small, no more than 0.5 kcal, and one is reluctant to draw conclusions from the actual conformations in the crystal since the energy of even a weak hydrogen bond would be considerably greater than the apparent barriers. In any case, two of the three possible rotamers are present (Figure 3). The torsion angles C8–N9–C11–C12 and C28–N29–C31–C32 (analogous to the χ angles of nucleosides) are 5.3 and 11.0°, respectively. Both independent molecules could be said to have the anti conformation as depicted in the diagram[29] for the molecular mechanics calculation on (S)-cytallene and as usually observed in structures of nucleosides with antiretroviral activity such as ddAdo.[35,36]

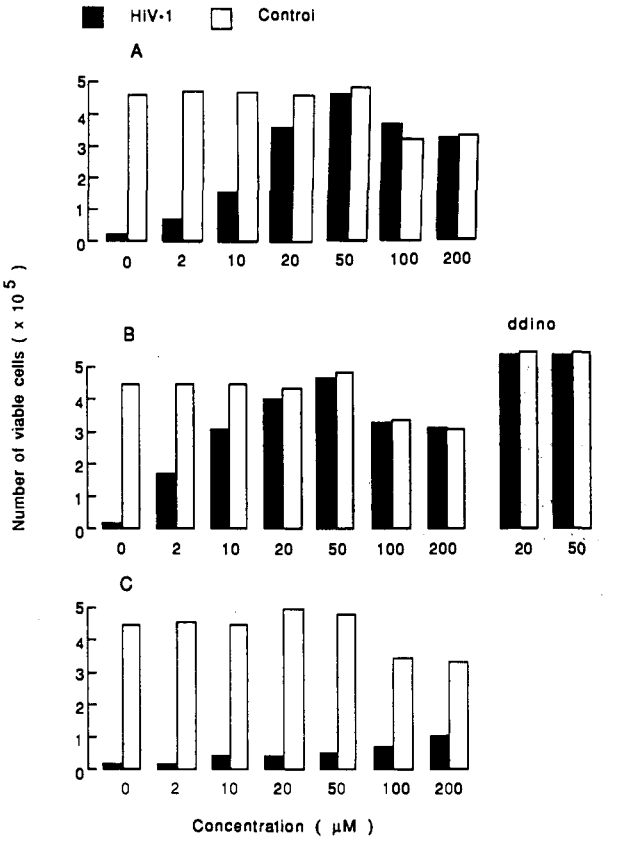


Figure 5. Inhibition of infectivity and cytopathic effect of HIV-1 in ATH8 cells by (±)-adenallene (**1a**), (R)-(−)-adenallene (**2**), and (S)-(+)-adenallene (**3**). The ATH8 cells were exposed to HIV-1 and incubated in the presence of (±)-adenallene (**1a**, panel A), (R)-(−)-adenallene (**2**, panel B), and (S)-(+)-adenallene (**3**, panel C, see Experimental Section). Infected cells are indicated as solid bars and control cells without the virus as open bars. (±)-Adenallene (**1a**, panel A) and ddIno in panel B served as positive controls.

Biological Activity

A. Inhibition of HIV-1. In view of the high anti-HIV activity of (±)-adenallene[10] (**1a**) it was of interest to study the inhibition of cytopathic effect and replication of HIV-1 with both enantiomers **2** and **3** (Figure 5) in a culture of ATH8 cells. Both 2′,3′-dideoxyinosine (ddIno in panel B), a recently approved drug for AIDS, and (±)-adenallene (**1a**, IC_{50} 14 μM, panel A) were used as positive controls. It is clear that *R-enantiomer 2 affords a significantly higher protection of the infected cells from the cytopathic effect of HIV-1 than S-enantiomer 3* (Figure 5, panels B and C). The respective IC_{50} values are 5.8 and >200 μM.

(28) Bruger, W. XICAMM, Computer Aided Molecular Modeling System, Version 2.0, 1986, Xiris Corp., Monmouth, NJ 07748.

(29) Taylor, E. W.; Van Roey, P.; Schinazi, R. F.; Chu, C. K. A Stereochemical Rationale for the Activity of Anti-HIV Nucleosides. *Antiviral Chem. Chemother.* **1990**, *1*, 163–173.

(30) Van Roey, P.; Taylor, E. W.; Chu, C. K.; Schinazi, R. F. Correlation of Molecular Conformation and Activity of Reverse Transcriptase Inhibitors. *Ann. N.Y. Acad. Sci.* **1990**, *616*, 29–40.

(31) In addition, the proposal[32] of 3′-exo conformation as a determinant of anti-HIV activity of nucleoside analogues is of limited validity.[33,34]

(32) Van Roey, P.; Salerno, J. M.; Chu, C. K.; Schinazi, R. F. Correlation Between Preferred Sugar Ring Conformation and Activity of Nucleoside Analogues against Human Immunodeficiency Virus. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3929–3933.

(33) Tseng, C. K.-H.; Marquez, V. E.; Milne, G. W. A.; Wysocki, R. J., Jr.; Mitsuya, H.; Shirasaki, T.; Driscoll, J. S. A Ring-Enlarged Oxetanocin A Analogue as an Inhibitor of HIV Infectivity. *J. Med. Chem.* **1991**, *34*, 343–349.

(34) Chu, C. K.; Ahn, S. K.; Kim, H. O.; Beach, J. W.; Alves, A. J.; Jeong, L. S.; Islam, Q.; Van Roey, P.; Schinazi, R. F. Asymmetric Synthesis of Enantiomerically Pure (−)-(1′*R*,4′*R*)-Dioxolane-thymine and Its Anti-HIV Activity. *Tetrahedron Lett.* **1991**, *32*, 3791–3794.

(35) Silverton, J. V.; Quinn, F. R.; Haugwitz, R. D.; Todaro, L. J. Structures of Two Dideoxynucleosides: 2′,3′-Dideoxyadenosine and 2′,3′-Dideoxycytidine. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1988**, *C44*, 321–324.

(36) Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. General Syntheses of 2′,3′-Dideoxynucleosides and 2′,3′-Didehydro-2′,3′-dideoxynucleosides. *J. Org. Chem.* **1989**, *54*, 2217–2225.

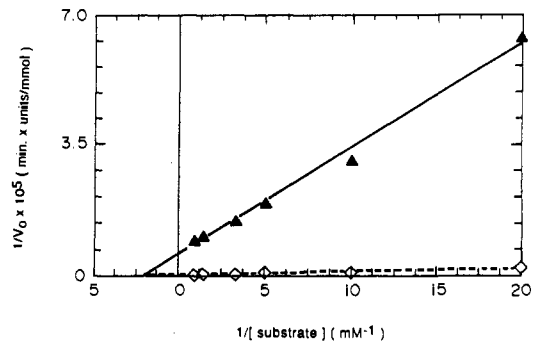


Figure 6. Lineweaver–Burk plot of deamination of (*R*)-(−)- and (*S*)-(+)-adenallene catalyzed by adenosine deaminase (ADA). (▲) (*S*)-(+)-Adenallene (**3**); (◇) (*R*)-(−)-adenallene (**2**). For details see Experimental Section.

The toxicity effects were apparent in experiments with racemic **1a** and enantiomers **2** or **3** alike at 100 and 200 μM. It is noteworthy that a less active enantiomer **3** is equally toxic as highly potent form **2**. In view of a high optical purity of *S*-enantiomer **3** contamination with a more active *R*-form **2** cannot explain the observed results. Thus, *S*-form **3** has its own, albeit lower, activity. The *enantioselectivity of (R)-adenallene (2) is lower than that of ddAdo.* Thus, L-enantiomer of the latter agent exhibited no activity[37] at 100 μM.[38]

B. Kinetics of Deamination with ADA. As indicated above, the different rates of deamination of *S*- and *R*-enantiomer can serve as a basis for their preparation in optically pure form. Generally, enzymic deamination is responsible for inactivation of biologically active adenosine analogues[41] in vivo. Therefore, investigation of the kinetics of deamination was necessary for assessing quantitatively its significance for metabolic deactivation of the most active *R*-form of adenallene **2**. It should be noted that deamination of ddAdo does not adversely influence its anti-HIV activity. In fact, the product of deamination (ddIno) is converted back to ddAdo in cytoplasm of the target cells[42] at the 5′-phosphate level. Such a "reamination" is apparently not possible in case of (±)-adenallene (**1a**) because racemic hypoxallene (**1d**) is devoid of anti-HIV activity.[10]

The kinetics of deamination of both enantiomers **2** and **3** was followed by HPLC, a method which was also used for monitoring the preparative reaction (Figure 2). The respective K_m and V_{max} values were readily obtained from a Lineweaver–Burk plot (Figure 6). The K_m's of enantiomers **3** and **2** are very close, 0.41 and 0.52 mM, respectively, indicating a similar binding affinity of both enantiomers for ADA. The corresponding V_{max} values are 530 and 18.5 μmol/min. Thus, as in case of HIV inhibition, a much lower enantioselectivity was observed as compared, e.g., with D- and L-adenosine.[2] Most surprisingly, *the enantioselectivities of deamination and anti-HIV effect are reversed.* In stark contrast to D-nucleosides which are invariably the most active enantiomers in biological assays, *R*-form **2** is more effective in inhibiting the HIV replication but less efficient in the ADA-catalyzed deamination. Both *R*- and *S*-forms **2** and **3** are substrates of only moderate activity. Consequently, the ADA-catalyzed deamination is of little significance for deactivation of *R*-enantiomer **2** except for higher concentrations. Thus, the IC_{50} value of **2** in an anti-HIV assay (Figure 5) is 90 times lower than the respective K_m of deamination.

The results presented here are of theoretical and practical interest. *Both (R)- and (S)-adenallene can function as analogues of adenine nucleosides but with a varied enantioselectivity for different enzymes (receptors).* It must also be emphasized that allenols **1** possess only a single chiral element (chiral axis) whereas 2′,3′-dideoxyribonucleosides comprise two centers of asymmetry. In addition, the fact that (*R*)-adenallene (**2**), a *direct* product of a controlled enzymic deamination of racemic **1a**, gives the most protection against the cytopathic effect of HIV but it is the least responsive toward enzymic deamination, may further increase its prospects for a potential therapeutic utilization.

Experimental Section

General Methods. See ref 2. High-performance liquid chromatography (HPLC) was run using a Bakerbond Chiral Phase Chiralcel CA-1 column (250 × 4.9 mm, J. T. Baker, Inc., Phillipsburg, NJ). The NMR spectra were measured in $(CD_3)_2SO$ unless stated otherwise. The electron-impact (EI-MS), chemical ionization (CI-MS), and fast-atom bombardment mass spectra (FAB-MS) were determined with Kratos MS80 RFA high resolution instrument. Thioglycerol was used as a matrix for FAB-MS. The optical rotations were determined with JASCO DIP-370 digital polarimeter. The circular dichroism (CD) spectra were measured using JASCO J-600 CD spectrometer. Adenosine deaminase (ADA from calf intestine, EC 3.5.4.4) was a product of Sigma Chemical Co., St. Louis, MO). For preparative deaminations Type II ADA was used whereas in kinetic experiments Type VIII was employed.

(*R*)-(−)-N^9-(4-Hydroxy-1,2-butadien-1-yl)adenine (2) and (*S*)-(+)-N^9-(4-Hydroxy-1,2-butadien-1-yl)hypoxanthine (4). A solution of (±)-adenallene[2] (**1a**, 1 g, 4.9 mmol) was stirred in 0.05 M Na_2HPO_4 (pH 7.5, 300 mL) at 26 °C (bath temperature). ADA (Type II, 85 mg, 153 units) in the same buffer (2 mL) was added, and the course of the reaction was monitored by HPLC on Chiralcel CA-1 column (20-μL aliquots were withdrawn and diluted with 1 mL of ethanol, and 20 μL was injected, see Figure 2). After 80 min, practically all (*S*)-(+)-adenallene (**3**) was converted to (*S*)-(+)-hypoxallene (**4**). The reaction was stopped by freezing the solution which was immediately lyophilized. The residue was washed several times with warm CH_2Cl_2–MeOH (4:1, 400 mL total). The solvents were evaporated, and the crude product was chromatographed on a silica gel column using CH_2Cl_2–MeOH (9:1) to give (*R*)-(−)-adenallene (0.39 g, 78 %): mp 172–173 °C after crystallization from ethyl acetate–MeOH (9:1); $[\alpha]^{25}_D$ −179° (MeOH, *c* 0.2); UV_{max} (EtOH) 262 nm (ε 14 700), 218 (ε 24 900); CD_{max} (pH 7) 235 nm ([θ] −23 100). The ^1H NMR spectra and EI-MS were identical with those of racemic[2] **1a**. Exact mass calcd 203.0808, found 203.0803. Anal. ($C_9H_9N_5O$) C, H, N.

Elution with CH_2Cl_2–MeOH (4:1) gave (*S*)-(+)-hypoxallene (**4**): 0.45 g (90 %); mp 227–229 °C after crystallization from 90 % EtOH; $[\alpha]^{25}_D$ 175° (MeOH, *c* 0.04); UV_{max} (pH 7) 226 nm (ε 22 800);

(37) Mansuri, M. M.; Farina, V.; Starrett, J. E., Jr.; Benigni, D. A.; Brankovan, V.; Martin, J. C. Preparation of the Geometric Isomers of DDC, DDA, D4C and D4T as Potential Anti-HIV Agents. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 65–68.

(38) L-AZT (L-3′-azido-3′-deoxythymidine) is ca. 10 000 times less potent than the D-enantiomer.[39] Results with 2′,3′-dideoxycytidine, another effective antiretroviral agent, are controversial. One report[40] indicated no activity of the L-form (no data was given) which was contradicted by results of a different study.[37]

(39) Wengel, J.; Lau, J.; Pedersen, E. B.; Nielsen, C. M. Synthesis of L-3′-Azido-3′-deoxythymidine and Its Stereoisomers. *J. Org. Chem.* **1991**, *56*, 3591–3594.

(40) Okabe, M.; Sun, R.-C.; Tam, S. Y.-K.; Todaro, L. J.; Coffen, D. L. Synthesis of the Dideoxynucleosides ddC and CNT from Glutamic Acid, Ribonolactone, and Pyrimidine Bases. *J. Org. Chem.* **1988**, *53*, 4780–4786.

(41) Plunkett, W. Inhibition of Adenosine Deaminase to Increase the Antitumor Activity of Adenine Nucleoside Analogues. *Ann. N.Y. Acad. Sci.* **1985**, *451*, 150–159.

(42) Johnson, M. A.; Ahluwalia, G.; Connelly, M. C.; Cooney, D. A.; Broder, S.; Johns, D. G.; Fridland, A. Metabolic Pathways for the Activation of the Antiretroviral Agent 2′,3′-Dideoxyadenosine in Human Lymphoid Cells. *J. Biol. Chem.* **1988**, *263*, 15354–15357.

CD_{max} (pH 7) 233 nm ([θ] 20 600). The ^1H and ^{13}C NMR spectra were identical with those of racemic[2] **1d**. Anal. ($C_9H_8N_4O_2$) C, H, N.

(S)-(+)-N^9-(4-Acetoxy-1,2-butadien-1-yl)hypoxanthine (5). Compound **4** (274 mg, 1.34 mmol) was made anhydrous by a coevaporation with pyridine (3 × 3 mL) in vacuo at room temperature. The residue was then stirred with acetic anhydride (760 μL) in pyridine (10 mL) at room temperature for 3 h. The clear solution was evaporated, and the crude product **5** was chromatographed on a silica gel column using CH_2Cl_2–MeOH (95:5): 271 mg (82%); mp 176–177 °C after crystallization from ethyl acetate–MeOH (9:1); $[\alpha]^{25}_D$ 205° (MeOH, *c* 0.04); UV_{max} (EtOH) 224 nm (ε 25 900); CD_{max} (EtOH) 231 nm (θ 26 000); ^1H NMR δ 12.44 (bs, 1 NH), 8.07 and 8.06 (2 s, 2, H_8 and H_2), 7.46 (m, 1 H, $H_{1'}$), 6.28 (q, 1, $H_{3'}$), 4.68 (m, 2 H, $H_{4'}$), 1.99 (s, H 3, Me); ^{13}C NMR 197.81 ($C_{2'}$), 170.64 (CO, acetate), 101.22 ($C_{3'}$), 95.06 ($C_{1'}$), 61.23 ($C_{4'}$), 21.09 (Me); hypoxanthine:[43] 157.00 (C_6), 147.79 (C_4), 146.93 (C_2), 138.43 (C_8), 124.97 (C_5); FAB-MS 247 (M + H, 87.9). Anal. ($C_{11}H_{10}N_4O_3$) C, H, N.

(S)-(+)-N^9-(4-Acetoxy-1,2-butadien-1-yl)adenine (6). Acetate **5** (225 mg, 0.91 mmol) was made anhydrous by coevaporation with pyridine (3 × 3 mL) whereupon it was suspended in CH_2Cl_2–pyridine (4:1, 10 mL) at −30 °C. Trifluoromethanesulfonic anhydride (230 μL, 1.37 mmol) was then added with stirring as a 10% solution in CH_2Cl_2. The mixture was then allowed to warm to a room temperature and the stirring was continued for 7 h. The resultant solution was added to dioxane saturated with NH_3 (10 mL), and the mixture was stirred at room temperature for 1 h. The solution was evaporated and crude **6** was chromatographed on a silica gel column using ethyl acetate–MeOH (97:3) to give 40 mg (22%): mp 173–175 °C after crystallization from ethyl acetate; $[\alpha]^{25}_D$ 190° (MeOH, c 0.04); UV_{max} (EtOH) 261 nm (ε 12 100), 213 (ε 25 900); CD_{max} (EtOH) 234 nm (θ 22 100); ^1H NMR δ 8.16 and 8.12 (2 s, 2, H_2 and H_8), 7.50 (m, 1 H, $H_{1'}$), 7.37 (s, 2, NH_2), 6.26 (q, 1, $H_{3'}$), 4.68 (m, 2, $H_{4'}$), 1.99 (s, 3, Me); ^{13}C NMR 197.51 ($C_{2'}$), 170.76 (CO, acetate), 101.04 ($C_{3'}$), 95.02 ($C_{1'}$), 61.36 ($C_{4'}$), 21.09 (Me); adenine:[43] 156.45 (C_6), 153.69 (C_2), 148.91 (C_4), 138.96 (C_8), 119.28 (C_5); FAB-MS 245 (M, 100.0). Anal. ($C_{11}H_{11}N_5O_2$) C, H, N.

(S)-(+)-N^9-(4-Hydroxy-1,2-butadien-1-yl)adenine (3). A. From Acetate 5. The reaction was performed as described above on a 1-mmol scale. The solvents were removed, the residue was dissolved in methanolic NH_3 (10 mL), and the resultant solution was stirred at room temperature for 7 h. Evaporation and chromatography on silica gel in ethyl acetate–MeOH (96:4) afforded enantiomer **3** (40 mg, 20%): mp 171–173 °C after crystallization from ethyl acetate–MeOH (9:1), $[\alpha]^{25}_D$ 181° (MeOH, *c* 0.2), UV_{max} (EtOH) 262 nm (ε 14 000), 216 (ε 24 400); CD_{max} (pH 7) 235 nm ([θ] 22 400). The ^1H NMR spectra and EI-MS were identical with those of enantiomer **2** and racemate[2] **1a**. Exact mass calcd 203.0808, found 203.0803. Anal. ($C_9H_9N_5O$) C, H, N.

B. Deacetylation of 6. A solution of compound **6** (27 mg, 0.11 mmol) in methanolic NH_3 (5 mL) was stirred for 1.5 h at room temperature. After evaporation, the residue was purified by chromatography on a silica gel column in CH_2Cl_2–MeOH (9:1) to give enantiomer **3** (20 mg, 90%): mp 171–172 °C after crystallization from ethyl acetate–MeOH (9:1), identical (UV, optical rotation) with the product obtained by method A.

(S)-(+)-6-Chloro-N^9-(4-acetoxy-1,2-butadien-1-yl)purine (7). Acetate **6** (90 mg, 0.375 mmol) was refluxed with (chloromethylene)dimethylammonium chloride (2 M, 375 μL, 0.75 mmol) in chloroform[21,22] (5 mL) for 1.5 h. Evaporation and chromatography of the residue on a silica gel column using CH_2Cl_2–MeOH (97:3) gave compound **7** (76 mg, 81%): mp 100 °C after crystallization from $CHCl_3$–petroleum ether (fraction 30–60 °C); $[\alpha]^{25}_D$ 166° (MeOH, *c* 0.2); UV_{max} (ethanol) 260 nm (ε 9 500), 222 (ε 28 700); CD_{max} (ethanol) 232 nm ([θ] 21 400); ^1H NMR ($CDCl_3$) δ 8.76 and 8.28 (2 s, 2, H_8 and H_2), 7.43 (m, 1, $H_{1'}$), 6.23 (q, 1, $H_{3'}$), 4.77 (t, 2, $H_{4'}$), 2.06 (s, 3, Me); CI-MS 265 (M + H, 100.0); exact mass calcd for M + H 265.0492, found 265.0497. Anal. ($C_{11}H_9ClN_4O_2$) C, H, Cl, N.

Crystal Data. A. Preliminary Information. The crystals for X-ray diffraction were obtained by a slow crystallization of (−)-adenallene (**2**) from ethanol. Yellowish prismatic crystal, 0.10 × 0.20 × 0.50 mm, Cu Kα radiation, λ = 1.54184 Å, Enraf-Nonius CAD4 diffractometer, graphite monochromator, monoclinic, *a* = 29.531 (2), *b* = 8.407 (1), *c* = 7.691 (1) Å, β = 91.69 (1)°, *V* = 1908.5 Å^3, *Z* = 4, fw = 203.20, ρ (calculated) = 1.41 g cm^{-3}, space group: *C*2 (no. 5), choice based on known optical activity, confirmed by successful refinement. For experimental methods, see ref 44.

B. Data Collection and Structure Solution. 23 (1) °C, 2θ (max): 148.0°, θ/2θ scan, 4 099 reflections (Friedel pairs where geometrically possible), total loss of standard intensity: 2.9%, automatic direct methods: *E* (minimum) of 1.5 and 1.2 both failed. Inspection showed no *E* values for any of the *h*0*l* planes with *l* odd needed for standard origin specification (such planes were observed if rather weak). Phase problem was solved by gradually increasing number of variable phases until convergence map was well-behaved. With 433 reflections (minimum *E* of 1.20) and 12 153 relationships, 99 phase sets, 18 atoms from *E* map prepared from best phase set, remaining atoms were located in difference map. Full-matrix least-squares refinement on $\sum w(|F_o| - |F_c|)^2$, H atoms included riding on bonded atoms, statistical weights and anomalous dispersion effects[45-47] included in F_c, 3 173 reflections with $I \geq 3\sigma(I)$, final cycle: 271 variables, parameters, largest shift: 0.02σ, *R* = 0.066, R_w = 0.093, no significant residual electron density in the final difference map (largest values: 0.5 and −0.3 e Å^3). All calculations were performed on a DEC 3520 Vax Station.[48]

C. Absolute Configuration. The possible enantiomers could not be distinguished by any of the standard significance tests. Refinement of the Flack x-parameter[49] was inconclusive but, given the pseudosymmetry and the presence of only one oxygen atom in each molecule, this failure was not surprising. The method of Engel[50] was then applied using the 23 reflections calculated to have the largest measurable Friedel differences. The original arbitrarily chosen enantiomer, depicted in Figure 3, proved most probable. The significance level is a little greater than 1 in 200 and the molecules, as shown in Figure 3, have the *R* configuration.[24] The asymmetric unit (two molecules) is shown in Figure 3, and full lists of refinement parameters are available in the supplementary material.

Inhibition of HIV Cytopathic Effect. The assay was performed with ATH8 cells as previously described.[10] The ATH8 cells (2 × 10^5) were exposed to HIV-1/III$_B$ also known as HIV-1 LA$_I$ (2000 virus particles/cell) for 45 min, resuspended in culture medium (2 mL) containing interleukin 2 in the presence or absence of various concentrations of allenols **1a**, **2**, and **3**. The mixtures were incubated in culture tubes at 37 °C in 5% CO_2/95% air humidified atmosphere. Control cells not exposed to the virus were treated similarly. On day 7 of the culture, the total viable cells were counted in a hemocytometer by the trypan blue exclusion method. The data are summarized in Figure 5.

Kinetics of Deamination with ADA. The assay was based on the method outlined above for a preparative experiment. Initial velocities (V_o) of (*R*)- and (*S*)-adenallene **2** and **3** were measured at six different concentrations of substrates: 1, 0.7, 0.3, 0.2, 0.1, and 0.05 mM and two enzyme concentrations (0.01 and 0.1 units,

(43) For assignments of heterocyclic signals see: Breitmaier, E.; Voelter, W. *^{13}C NMR Spectroscopy*; Verlag Chemie: New York, 1987; p 405 (Table 5.22).

(44) CAD4 Operations Manual, Enraf-Nonius: Delft, Holland, 1977.
(45) Cromer, D. T. *International Tables for X-Ray Crystallography*; Kynock Press: Birmingham, England, 1974; Vol. IV, Table 2.3.1.
(46) Hutcheon, W. L. B.; James, M. N. G. Molecular Geometry of 2',3'-Dideoxy-2',3'-didehydroadenosine from an X-ray Crystal Structure Determination. *Acta Crystallogr., Sect. B: Struct. Sci.* **1974**, *B30*, 1777–1782.
(47) Ibers, J. A.; Hamilton, W. C. Dispersion Corrections and Crystal Structure Refinements. *Acta Crystallogr.* **1964**, *17*, 781–782.
(48) MolEN. An Interactive Structure Solution Procedure; Enraf-Nonius: Delft, Holland, 1990.
(49) Bernardinelli, G.; Flack, H. D. Least-Squares Absolute-Structure Refinement. Practical Experience and Ancillary Calculations. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1985**, *A41*, 500–511.
(50) Engel, D. W. The Determination of Absolute Configuration and Δf'' Values for Light-Atom Structures. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1972**, *B28*, 1496–1509.
(51) IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Abbreviations and Symbols for Nucleic Acids, Polynucleotides and Their Constituents. *Biochim. Biophys. Acta* **1971**, *247*, 1–12.

respectively) in a total volume of 3 mL of 0.05 M Na_2HPO_4 (pH 7.5). The reactions were initiated by adding enzyme solution to the mixture preequilibrated at 26 °C. Aliquots of 50 μmol were withdrawn at 15-min intervals and analyzed by HPLC as described above. Calibration of the column was performed with the same solutions of substrates without the enzyme. Each standard was analyzed three times, and the average integration of peaks was used. The V_0 was determined for each experiment, and saturation curves were constructed by plotting V_0 vs substrate concentration. The K_m and V_{max} were obtained from the corresponding Lineweaver–Burk (double-reciprocal) plot (Figure 6).

Acknowledgment. Thanks are due to the Central Instrumentation Facility (Department of Chemistry, Wayne State University, Dr. Robin J. Hood, Director) and, particularly, Dr. M. Ksebati and M. Kempf for NMR and mass spectra. The work at the Michigan Cancer Foundation was supported in part by U.S. Public Health Service Research Grant CA32779 from the National Cancer Institute, Bethesda, MD and in part by an institutional grant to the Michigan Cancer Foundation from the United Way of Southeastern Michigan. Joel Orlina thanks the Heart Association of Montgomery County, MD for the award of a High School Honors Fellowship during the summer of 1991.

Supplementary Material Available: Crystallographic data in Tables I–VIII (10 pages). Ordering information is given on any current masthead page.