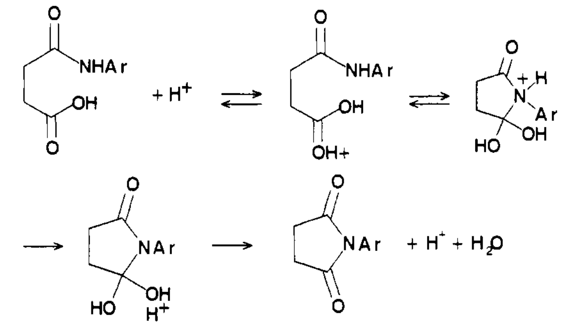
Scheme III



This gives a value for the second-order rate constant for acid catalysis in formation of MSI, $k_{1H'} = 1.1 \times 10^{-4}\ M^{-1}\ s^{-1}$.

In Table I, the value of log k_{obsd} for the apparent hydrolysis of MSA versus pH increases slightly at acidities greater than pH 3. This is due to the significance of $k_{1H'}$ for imide formation, which is not detected separately. The value of k_{2H} is insignificant at this acidity. The values of $k_{1H'}$ in this region can be calculated by extrapolation of the solid line in Figure 3 into the pH region. The values for k_2 (corrected for the rate of imide formation) are plotted in Figure 4.

Undissociated amic acids form anhydrides much more rapidly than do their conjugate bases.[1-5,10] The kinetic data plotted in Figure 4 follow the form of a titration curve with K_A equal to that of the carboxylic acid:

$$k_{obsd} = k_2/(1 + K_{A'}/[H^+]) \quad (7)$$

The data were fit to eq 7, which gives $k_2 = 1.6 \times 10^{-5}\ s^{-1}$ and a p$K_{A'}$ of 4.45 for MSA. The p$K_{A'}$ of the unsubstituted succinanilic acid has been determined by titration to be 4.4 (0.5 M NaCl, 25 °C).[18]

MSA partitions equally toward the imide MSI (Scheme II), and the hydrolysis products at the acidity in which the apparent rate constants are equal.

$$k_{1obsd} = k_{1H} \quad (8)$$

$$k_{2obsd} = k_2 + k_{2H} \quad (9)$$

Thus k_{1obsd} is equal to k_{2obsd} at "pH" 0.78.

Mechanism of Imide Formation. Acid-catalyzed formation of an imide from an amide can be formulated to occur by the mechanism in Scheme III. Protonation of the carboxyl group is followed by attack of the amide nitrogen at the carboxyl carbon, forming a protonated tetrahedral intermediate. Proton transfer and elimination of water produces the imide. Hydrolysis of the imide occurs by the reverse reaction, followed by hydrolysis of the amic acid.

The hydrolysis of MSA has a much weaker acid-catalysis component than does the formation of the imide from MSA. This small rate component is most likely due to the direct reaction of water with MSA and not the reaction in which carboxyl participation is involved. The rate constant is consistent with expectations for the reaction of a simple amide in strong acid solution.

Conclusions

The well-known rapid hydrolysis of amic acids in acidic solution involves a transacylation reaction, which potentially competes initially with a dehydration reaction leading to formation of an imide. The pseudoequilibrium between amic acid and imide is drained by the conversion of the amic acid to the amine and dicarboxylic acid via the anhydride. This complication in the behavior of amic acids indicates that changes in the initial spectrum of amic acids in acidic solution do not necessarily indicate the formation of hydrolysis products.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for continued support through an operating grant.

Nucleic Acid Derived Allenols: Unusual Analogues of Nucleosides with Antiretroviral Activity[1]

Shashikant Phadtare and Jiri Zemlicka*

Contribution from the Department of Chemistry, Michigan Cancer Foundation, and the Department of Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan 48201. *Received June 30, 1988*

Abstract: Racemic 1,2-butadien-4-ols substituted with a nucleic acid base were prepared by a base-catalyzed isomerization of the corresponding 2-butynols. With basic heterocycles such as adenine, cytosine, 5-methylcytosine, or *N*-[(dimethylamino)methylene]guanine, the respective allenes were obtained without difficulty, but with guanine, side reactions were observed. Reaction of 2-butynols in stronger base (1 M NaOH) gave cyclized products—oxacyclopentenes **8a–c**. (±)-Adenallene (**3a**) and (±)-cytallene (**3c**) are strong inhibitors of replication of human immunodeficiency virus (HIV) in vitro. (±)-Adenallene (**3a**) and butyne **6a** are substrates for adenosine deaminase. Racemic **3a** was deaminated quantitatively to (±)-hypoxallene (**3h**), indicating a low stereoselectivity as contrasted with the natural substrate—adenosine. When the deamination was stopped at ca. 50% conversion, (−)-adenallene (**3a**) and (+)-hypoxallene (**3h**) were obtained. Antiretroviral and adenosine deaminase substrate activities are discussed in terms of the similarity of several steric and stereoelectronic features of allenic derivatives of nucleic bases with those of the corresponding nucleosides or 2′,3′-dideoxyribonucleosides.

Nucleoside analogues are the center of current interest as antiviral chemotherapeutic agents. Especially important are "acyclic" analogues, which can be formally derived by a cleavage of one or more bonds of the furanose ring. Thus, structure **1a** is an acyclic analogue of adenosine lacking the $C_{2'}$ and $C_{3'}$ atoms. Both adenine[2] and guanine derivatives **1a** and **1b** are biologically active; indeed, the latter is a clinically useful antiherpetic drug, acyclovir.[3] Similarly, analogue **2a** relates to the antibiotic aristeromycin,[5] and the corresponding guanine derivative **2b** is an

(1) Various aspects of this work were presented at or in the following: 7th Symposium on the Chemistry of Nucleic Acid Components, Aug 30 to Sept 5, 1987, Bechyne Castle, Czechoslovakia. Phadtare, S.; Zemlicka, J. *Nucleic Acids Symp. Ser.* **1987**, *No. 18*, 25. 3rd Chemical Congress of North America, June 5–10, 1988, Toronto, Ontario, Canada; Abstract MEDI 24. 15th Symposium on Nucleic Acid Chemistry, Sept 19–21, 1988, Sapporo, Japan. Phadtare, S.; Zemlicka, J. *Nucleic Acids Symp. Ser.* **1988**, *No. 20*, 39.

(2) Schaeffer, H. J.; Gurwara, S.; Vince, R.; Bittner, S. *J. Med. Chem.* **1971**, *14*, 367.

(3) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716.

(4) Schaeffer, H. J.; Vogel, D. *J. Med. Chem.* **1965**, *8*, 507.

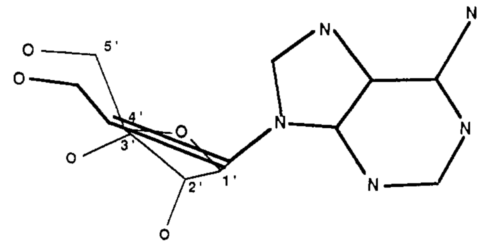


Figure 1. Computer-generated overlap of adenosine (3′-endo form) with (*S*)-adenallene (**3a**). Overlap was determined by using Computer Aided Molecular Modeling System (XICAMM), Xiris Corp., New Monmouth, NJ. Only the ribofuranose portion is numbered. All hydrogen atoms were omitted for clarity. Overlap of purine rings is virtually total. Cumulated system of double bonds of **3a** is shown as a single double bond. Note proximity of π orbitals of allenic portion (**3a**) with p orbitals of $O_{4'}$ (adenosine) as well as that of $O_{5'}$ (adenosine) and the corresponding oxygen atom of **3a**. According to a referee, the distance between C-2′ (**3a**) and O-4′ (adenosine) is 0.9 Å.

Chart I. Definition of Base B in Structures **1–12**

series **a**: B = adenin-N^9-yl
series **b**: B = guanin-N^9-yl
series **c**: B = cytosin-N^1-yl
series **d**: B = 5-methylcytosin-N^1-yl
series **e**: B = 2-amino-6-chloropurin-N^9-yl
series **f**: B = N^2-[(dimethylamino)methylene]guanin-N^9-yl
series **g**: B = N^6-[(dimethylamino)methylene]adenin-N^9-yl
series **h**: B = hypoxanthin-N^9-yl

antiviral agent.[6] (See Chart I for definition of base B.)

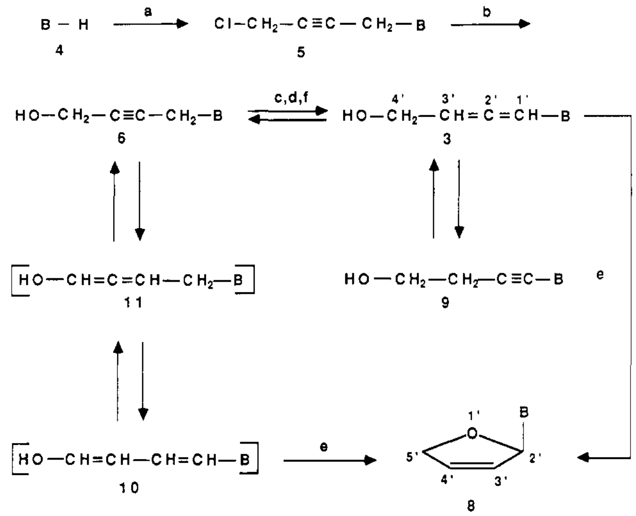
In vivo phosphorylation is of paramount importance for biological activity of many nucleoside analogues[7] including some acyclic derivatives.[8] Whereas a structural relationship of acyclovir (**1b**) to guanosine is obvious, we have become interested in acyclic

$$\underset{\mathbf{1}}{HOCH_2CH_2OCH_2B} \qquad \underset{\mathbf{2}}{HOCH_2CH_2CH_2CH_2B}$$

compounds with a less explicit likeness to parent nucleosides but with sufficient common features to function as analogues thereof. Thus, a computer-generated molecular overlap (Figure 1) indicated some similarities between adenosine in 3′-endo conformation[9] and the corresponding allene **3a**. It is clear that both key elements, hydroxymethyl functions and adenine rings, can attain similar positions when bound to a receptor. In addition, the position of π orbitals of cumulated double bonds approximates that of p orbitals of the ring oxygen atom of the ribofuranose moiety. The similarity of both molecules becomes even more apparent when the 2′- and 3′-hydroxy groups are replaced with hydrogen atoms to give 2′,3′-dideoxyadenosine. The class of 2′,3′-dideoxyribonucleosides is currently receiving considerable attention,[13] owing to their activity against human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS). It seemed, therefore, likely that allenic derivatives of nucleic acids carrying a suitably oriented hydroxymethyl function may be viewed as analogues of 2′,3′-dideoxyribonucleosides.

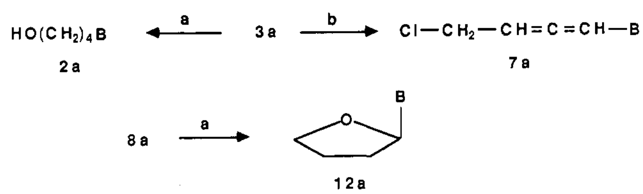
These considerations led us to investigate synthetic avenues to such allenes. Simple allenic derivatives of some unfunctionalized

Scheme I[a]



[a] (a) 1,4-Dichloro-2-butyne; K_2CO_3; DMSO. (b) 0.1 M HCl; Δ. (c) 0.1 M NaOH; Δ. (d) Potassium *tert*-butoxide; DMF. (e) 1 M NaOH; dioxane–H_2O (1:1); Δ. (f) 0.1 M NaOH; dioxane–H_2O (1:4); Δ.

Scheme II[a]



[a] (a) H_2; Pd/C; ethanol. (b) $(C_6H_5)_3P$; CCl_4; DMF.

heterocyclic systems (pyrazole, indole, etc.) have been prepared in varying degrees of purity[14] by acetylene–allene isomerization.[15] The only allenes derived from a nucleic acid base, and briefly mentioned in the literature, are 5-allenyluracil and the corresponding 2′-deoxyribofuranoside.[16] The acetylene–allene isomerization approach proved to be the method of choice for our purposes.

Synthesis. Adenine (**4a**) was smoothly alkylated with 1,4-dichloro-2-butyne (4-fold excess) and K_2CO_3 in dimethyl sulfoxide (DMSO) to give the corresponding N^9-alkyladenine **5a** in 50% yield and >90% N^9/N^7 regioselectivity (Scheme I). The latter was readily hydrolyzed to butyne **6a** in almost 70% yield. In a similar fashion, alkylation of unprotected cytosine (**4c**) and 5-methylcytosine (**4d**) followed by hydrolysis led to the corresponding acetylenes **6c** and **6d** in 30% overall yield. An approach to guanine derivative **6b** proceeds from 2-amino-6-chloropurine (**4e**), which was alkylated with 1,4-dichloro-2-butyne. Subsequent acid hydrolysis of both chloro functions of **5e** gave the required acetylene derivative **6b** in 28% overall yield.[17]

The butyne **6a** was isomerized to the corresponding allene **3a** in 0.1 M NaOH at 100 °C for 30 min (Scheme I). The resultant mixture of isomers containing ca. 50% of allene **3a** was resolved by chromatography on silica gel. Adenallene[18] (**3a**) was obtained

(5) Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K. *J. Antibiot.* **1968**, *21A*, 255.
(6) Larsson, A.; Alenius, S.; Johansson, N. G.; Oberg, B. *Antiviral Res.* **1983**, *3*, 77.
(7) Bloch, A. In *Drug Designs*; Ariens, E. J., Ed.; Academic Press: New York, 1974; Vol. IV; p 286, loc cit 345.
(8) Keller, P. M.; Fyfe, J. A.; Beauchamp, L.; Lubbers, C. M.; Furman, P. A.; Schaeffer, H. J.; Elion, G. B. *Biochem. Pharmacol.* **1981**, *30*, 3071.
(9) It is generally agreed that the sugar conformation of nucleosides can be described as a 2′-endo ⇌ 3′-endo equilibrium.[10] The 3′-endo conformer is usually preponderant in both solution and solid state.[11] Other puckering modes have been observed in various forms of DNA[10] and in complexes of nucleotides with enzymes, e.g., DNA polymerase.[12]
(10) Saenger, W. *NATO ASI Ser., Ser. A* **1979**, *26*, 1.
(11) Danyluk, S. S. NATO ASI Ser., Ser. A **1979**, *26*, 15.
(12) Ferrin, L. J.; Mildvan, A. S. *Biochemistry* **1986**, *25*, 5131.
(13) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *83*, 1911.
(14) Hubert, A. J.; Reimlinger, H. *J. Chem. Soc. C* **1968**, 606.
(15) Landor, P. D. In *The Chemistry of the Allenes*; Landor, S. R., Ed.; Academic Press: New York, 1982; Vol. 1, p 21, loc cit 131.
(16) Bobek, M. 1st Pan American Chemical Congress, Oct 13–18, 1985, San Juan, Puerto Rico; Abstract 0320-1.
(17) According to a recent patent, compound **6b** was obtained by a different method in 14% overall yield: Johansson, K. N.-G.; Lindborg, B. G.; Noren, J.-O. European Patent 146 516, Bulletin 85/26, 1985, p 38.
(18) Suggested trivial names are based on nomenclature of nucleic acid bases and the suffix allene: adenine, adenallene; cytosine, cytallene; guanine, guanallene; hypoxanthine, hypoxallene; uracil, urallene; thymine, thymallene. Allenic compounds described herein and their antiretroviral activity are the subject of a joint patent application by the Michigan Cancer Foundation and National Cancer Institute.
(19) Schaeffer, H. J.; Bhargava, P. S. *Biochemistry* **1965**, *4*, 71.

in 33% yield. It is interesting that a more favorable allene/acetylene ratio was obtained when the isomerization was conducted with potassium *tert*-butoxide in dimethylformamide (DMF) at −15 °C for 1.5 h; adenallene (**3a**) was obtained in 50% yield. Hydrogenation of **3a** gave smoothly the known[4,19] N^9-(4-hydroxybutyl)adenine (**2a**) in 95% yield whereas chlorination with CCl_4 and $(C_6H_5)_3P$ afforded the respective 4′-chloro-4′-deoxyadenallene (**7a**) in 90% yield (Scheme II).

With stronger base (1 M NaOH in 50% dioxane at 100 °C for 1 h) butyne **6a** was converted to oxacyclopentene **8a** in 20% yield (Scheme I). This conversion provides a striking example how an open-chain intermediate can be converted into a nucleoside-like structure, and it is also of mechanistic interest. Thus, a similar cyclization of simple allenic alcohols[20,21] was classified as a favored 5-endo-dig process[22] although the reaction occurred at a trigonal carbon atom and in a strict sense[23] it should be regarded as a disfavored[22-24] 5-endo-trig reaction. In our case, likely intermediates include allene **3a** and the corresponding isomerization product **10a** formed via hydroxyallene **11a** (Scheme I). Nevertheless, cyclizations of both **3a** and **10a** are disfavored 5-endo-trig reactions. Hydrogenation of **8a** afforded the known[25] (tetrahydrofuryl)adenine **12a** in 90% yield (Scheme II).

Isomerization of cytosine acetylene derivative **6c** was achieved under similar conditions (Scheme I). Thus, heating in 0.1 M NaOH (20% dioxane) at 100 °C for 9 h gave a mixture containing **3c** and **6c**. The latter compound was isolated by chromatography and fractional crystallization in 30% yield. It is noteworthy that by using potassium *tert*-butoxide in DMF (overnight at room temperature) we have obtained an 80% yield of cytallene[18] (**3c**). This product contained according to the HPLC **3c** (73%), **6c** (13%), and a third component tentatively identified as 1-butyne **9c** (14%). Pure **3c** was obtained after four crystallizations from methanol in 39% yield. The 5-methylcytallene (**3d**) was prepared in the same fashion. In this case pure **3d** was obtained after chromatography in 25% yield. As in the case of acetylene **6a**, cyclization of **6c** in 1 M NaOH (50% dioxane, reflux for 1 h) gave oxacyclopentene **8c** in almost 40% yield.

Isomerization of guanine acetylene **6b** proved more difficult. Thus, heating with 0.1 M NaOH in 40% dioxane for 14 h gave only a 10% yield of an allene–acetylene mixture in the ratio of 35:65, which was not possible to resolve either by chromatography or crystallization. In stronger base (1 M NaOH) butyne **6b** was transformed to oxacyclopentene **8b** in 27% yield. Attempted isomerization with potassium *tert*-butoxide in DMF gave solely decomposition products. In view of the successful results with adenine and cytosine 2-butynes **6a**, **6c** and **6d**, it was tempting to rationalize this difficulty in terms of higher acidity of guanine. Thus, the CONH function becomes ionized in a strongly basic reaction mixture, and it can interact with the reactive allene system. Indeed, when the amino group of butyne **6b** was substituted with the *N*-[(dimethylamino)methylene] function,[26] giving a less acidic derivative, **6f**, isomerization with potassium *tert*-butoxide in DMF gave a 50% yield of a mixture containing 80% of allene **3f**. Crystallization of this material did not improve the allene/acetylene ratio, but deprotection with NH_3 in methanol, chromatography, and subsequent crystallization afforded guanallene[18] (**3b**) in 90% purity and 55% yield.

Structure of Products. Spectroscopic methods were invaluable in the confirmation of structures of the allenic derivatives and transformation products thereof. Most, if not all known allenes, exhibit a characteristic ν_{as} in the IR spectra[27] of the C=C=C grouping near 1950 cm^{-1}. It is therefore surprising that allenes **3a**, **3b**, and **3h** fail to show this peak altogether whereas cytosine derivatives **3c** and **3d** exhibit only weak bands between 1960 and 1970 cm^{-1}. Only chloroallene **7a** exhibits a well-developed ν_{as} at 1961 cm^{-1}. By contrast, the IR spectra of 2,3-butadien-1-ol[28] and *N*-allenyl heterocycles[14] include bands at 1961 cm^{-1}. At present, it is difficult to explain this observation, but hydrogen-bonding effects of hydroxy groups as well as nonbonding interactions of nucleobases with the π orbitals of the allene system could play some role.

The NMR spectroscopy was of greater value for characterization of the allenes. Thus, the $C_{2'}$ signals in the ^{13}C NMR of **3a**, **3c**, **3d**, **3h**, and **7a** are between 194 and 198 ppm in good accord with other simpler derivatives.[29] In addition, characteristic olefinic signals ($H_{1'}$ and $H_{3'}$) are found in the ^1H NMR spectra. The position of the $H_{1'}$ (δ 7.1–7.6) corresponds to that found for a similar signal in *N*-allenyl heterocycles[14] (δ ca. 7) lacking a hydroxymethyl function. It is also interesting that the UV spectrum of adenallene (**3a**) resembles that of N^9-vinyladenine.[30] Thus, the $C_{2'}$–$C_{3'}$ π-bond located perpendicularly to that of $C_{1'}$–$C_{2'}$ cannot effectively influence the conjugation of the latter with the adenine ring.

Structure assignment of oxacyclopentenes **8a–c** followed unequivocally from NMR and mass spectra. The chemical shifts of $H_{2'}$, $H_{3'}$, and $H_{4'}$ are close to those of the corresponding protons of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides.[31-33] As expected, the $H_{4'}$ are not magnetically equivalent, and they form two sets of multiplets at δ ca. 4.8 and 4.6. The mass spectra have shown, in addition to peaks of heterocyclic bases, a characteristic fragment for the oxacyclopentene moiety of m/z 69 and its decomposition products at m/z 68 (furan) and m/z 39 (see ref 34).

Electron-impact mass spectra of allene derivatives and their precursors reported herein were in agreement with the proposed structures. Their detailed analysis will be reported elsewhere.

Biological Activity. Preliminary biological tests of allenes **3a–d** in murine leukemia L1210 cell culture and several other antitumor and antiviral assays did not reveal significant biological activity. By contrast, adenallene (**3a**) and cytallene (**3c**) exhibit a strong antiretroviral effect against human immunodeficiency viruses HIV-1 and HIV-2 in vitro.[35] Their activities are commensurable with that of the anti-AIDS drug AZT (3′-azido-3′-deoxythymidine, zidovudine, retrovir) or with such 2′,3′-dideoxyribonucleosides as 2′,3′-dideoxyadenosine (ddAdo) and 2′,3′-dideoxycytidine (ddCyd), which are currently undergoing clinical testing. A possible analogy with the latter compounds is underlined by the fact that cytallene (**3c**) gives almost complete cell protection against infection at 0.5–1 μM whereas adenallene (**3a**) exhibited an equal effect at 50–100 μM and 5-methylcytallene (**3d**) was inactive. A similar trend of antiretroviral effect was observed with ddCyd, ddAdo, and 5-methyl-ddCyd, respectively.[13,36] A surprising selectivity for retroviruses is an additional feature which relates **3a** and **3c** to 2′,3′-dideoxyribonucleosides. Guanallene (**3b**) and hypoxallene[18] (**3h**) were both devoid of anti-HIV activity,[35] which is in a striking contrast to the significant activity of the corresponding 2′,3′-dideoxyribonucleosides.[13] In the case of 2′,3′-dideoxyinosine (ddIno), this effect was linked to an intracellular transformation to ddAdo

(20) Hoff, S.; Brandsma, L.; Arens, J. F. *Recl. Trav. Chim. Pays-Bas* **1969**, *88*, 609.
(21) Grange, D.; Magnus, P. *J. Am. Chem. Soc.* **1978**, *100*, 7746.
(22) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734.
(23) Baldwin, J. E.; Cutting, J.; Dupont, W.; Kruse, L.; Silberman, L.; Thomas, R. C. *J. Chem. Soc., Chem. Commun.* **1976**, 736.
(24) Baldwin, J. E.; Thomas, R. C.; Kruse, L. I.; Silberman, L. *J. Org. Chem.* **1977**, *42*, 3846.
(25) Lewis, L. R.; Schneider, R. H.; Robins, R. K. *J. Org. Chem.* **1969**, *26*, 3837.
(26) Zemlicka, J.; Holy, A. *Collect. Czech. Chem. Commun.* **1967**, *32*, 3159.
(27) Runge, W. In *The Chemistry of the Allenes*; Landor, S. R., Ed.; Academic Press: New York, 1982; Vol. 3, p 780.
(28) Crombie, L. E.; Jenkins, P. A.; Mitchard, D. A. *J. Chem. Soc., Perkin Trans. 1*, 1975, 1081.
(29) Ref 27, p 833.
(30) Kaye, H. *Polym. Lett.* **1969**, *7*, 1.
(31) McCarthy, J. R.; Jr.; Robins, M. J.; Townsend, L. B.; Robins, R. K. *J. Am. Chem. Soc.* **1966**, *88*, 1549.
(32) Russell, A. F.; Moffatt, J. G. *Biochemistry* **1969**, *8*, 4889.
(33) Horwitz, J. P.; Chua, J.; Noel, M.; Donatti, J. T. *J. Org. Chem.* **1967**, *32*, 817. Assignments of $H_{2'}$ and $H_{3'}$ of 2′,3′-dideoxy-2′-cytidinene were inadvertently interchanged.
(34) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. *Mass Spectrometry of Organic Compounds*; Holden-Day: San Francisco, 1967; p 615.
(35) For a detailed description of these results see: Hayashi, S.; Phadtare, S.; Zemlicka, J.; Matsukura, M.; Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6127.
(36) Kim, C.-H.; Marquez, V. E.; Broder, S.; Mitsuya, H.; Driscoll, J. S. *J. Med. Chem.* **1987**, *30*, 862.

at the 5′-monophosphate level.[37] Both ddAdo and ddIno are also substrates for enzymes of purine metabolism such as adenosine deaminase (ADA)[37,38] or purine nucleoside phosphorylase (PNP).[39] It was, therefore, of interest to compare the substrate activity of allene derivatives with those of 2′,3′-dideoxyribonucleosides. Thus, in contrast to ddIno,[39] hypoxallene (**3h**) is not a substrate for PNP, but adenallene (**3a**) and its precursor **6a** (inactive against HIV) are both substrates for ADA. Their substrate activities are relatively weak, at concentrations substantially above the levels sufficient for HIV inhibition. It is unlikely then that deamination of **3a**, catalyzed by ADA, contributes to any significant extent to its catabolism. Nevertheless, hydrolysis of **3a** catalyzed by ADA is of both theoretical importance and preparative utility. Thus, *racemic* adenallene (**3a**) was converted to *racemic* hypoxallene (**3h**) in *90%* isolated yield (deamination was virtually *quantitative*). This indicates a surprisingly low stereoselectivity as contrasted with natural substrates (D-adenosine).[40] An inspection of molecular models and computer-assisted overlaps of both enantiomers of adenallene (**3a**) suggests that the restriction for binding of an "unnatural" enantiomer of **3a** may be less severe than that for L-adenosine. Hence, a lesser stereoselectivity can be expected in case of **3a**. In another experiment, deamination of racemic **3a** was allowed to proceed to ca. 50% completion to give (+)-hypoxallene (**3h**, 30%) and (−)-adenallene (**3a**, 45%). This is, to the best of our knowledge, the first (partial) resolution of a heterocyclic allene, and its success contrasts with futile attempts to achieve differentiation of ^{1}H NMR signals by means of complexation of racemic **3g** with (+)-tris[3-[(trifluoromethyl)-hydroxymethylene]camphorato]europium(III) [Eu(tfc)$_3$]. We have not yet determined the optical purity of both products, which exhibit opposite signs of optical rotations and CD maxima. At this time, it is also not possible to assign absolute configurations to both enantiomers. Assignment of an *S* configuration for (+)-hypoxallene (**3h**) on the basis of Lowe's rule[43] may well be fortuitous. Nevertheless, it is of interest that (*S*)-9-(2,4-dihydroxybutyl)adenine,[44] a compound with a single center of chirality, was also a substrate for ADA whereas the *R* enantiomer was not deaminated.

The 2′,3′-dideoxyribonucleosides ddAdo and ddCyd differ in their response to enzymic deamination. Thus, the latter is not deaminated with cytidine deaminase[45] (CDA). Cytallene (**3c**) is also not a substrate for CDA. Because the 5′-hydroxy group[38] is essential for ADA binding whereas a corresponding role in CDA-catalyzed deamination is fulfilled[45] by the 3′-OH, it is not surprising that **3c** is resistant whereas **3a** is a substrate. By contrast, both analogues function well when they approximate the $C_{1'}$–$C_{5'}$ fragment of a nucleoside as, presumably, in HIV inhibition.

A further comparison of the requirements for ADA, an enzyme whose depletion can cause an immunodeficiency disease,[46] and HIV inhibition is of interest. As stated above, both **3a** and **6a** are substrates for ADA, but only **3a** is an anti-HIV agent. In addition, compound **2a** having a straight hydrocarbon chain is

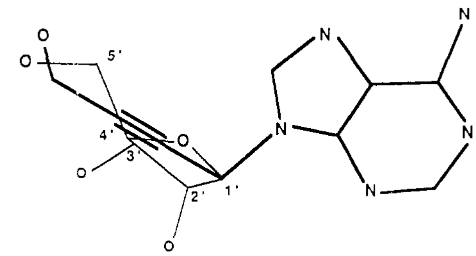


Figure 2. Computer-generated overlap of adenosine with butyne **6a**. See Figure 1 legend. Although both oxygens (hydroxymethyl groups) are close, overlap of the relevant π and p orbitals is poor.

inactive in both systems. It would then appear that the position of the heterocyclic base and hydroxy group along with the presence of a suitably located p or π orbital (ether oxygen or multiple bond) is important for HIV inhibition. It is evident from Figure 1 that π orbitals of the allene moiety occupy a position very similar to that of p orbitals of the furanose ring oxygen atom of adenosine. Cumulated double bonds may enhance the substrate binding either by hydrogen bonding from a receptor of π electrons[47] or by covalent interaction[48] with a suitable enzyme site (irreversible inhibition). In addition, an allenic system of double bonds is considerably more rigid than the fairly flexible furanose ring of 2′,3′-dideoxyribonucleosides. In this respect, cytallene (**3c**) and adenallene (**3a**) may resemble 2′,3′-didehydro-2′,3′-dideoxyribonucleosides, which also exhibit antiretroviral activity.[49,50] It then appears that conformational rigidity, location of base and hydroxy functions, and orientation of π orbitals all contribute to anti-HIV activity of adenallene (**3a**) and cytallene (**3c**). Interestingly, both inactive analogues **6a** and **2a** either do not provide an efficient overlap of π orbitals with p electrons of ribofuranose (Figure 2) or lack π electrons in the side chain entirely.

It would then appear that requirements for ADA substrate activity are more flexible (both **3a** and **6a** are substrates), but the presence of p or π orbitals, a factor hitherto unrecognized for ADA, is also important. Thus, unlike[2] **1a**, compound **2a** is not deaminated, and aristeromycin, a carbocyclic analogue of adenosine, is a substrate of low activity.[51]

Cytallene (**3c**) and adenallene (**3a**) are the first derivatives of nucleic acid bases with an antiretroviral activity which lack an oxacyclopentane[52] or equivalent (oxetane[53]) moiety comprising an ether oxygen atom. They are also the first rationally designed acyclic nucleoside analogues with an anti-HIV activity.[54] The allenic derivatives described herein are novel nucleoside analogues where the chiral centers of the ribofuranose portion are replaced with the chiral axis of a 1,3-disubstituted allene. It is possible that enantiomers of **3a** and **3c** may exhibit different levels of antiretroviral activity. To the best of our knowledge, cytallene (**3c**) and adenallene (**3a**) are also the first biologically active analogues of centrochiral systems based on a principle of axial chirality. This new approach may well become of more general applicability for the design of structures of biochemical and medicinal interest.

(37) Ahluwalia, G.; Cooney, D. A.; Mitsuya, H.; Fridland, A.; Flora, K. P.; Hao, Z.; Dalal, M.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1987**, *36*, 3797.

(38) Bloch, A.; Robins, M. J.; McCarthy, J. R., Jr. *J. Med. Chem.* **1967**, *10*, 908.

(39) Stoeckler, J. D.; Cambor, C.; Parks, R. E., Jr. *Biochemistry* **1980**, *19*, 102.

(40) In fact, L-adenosine is very slowly deaminated ($t/2$ ca. 6 days) but, surprisingly, faster than D-nucleoside tubercidin.[41] The latter is completely intact after 8 days. For a similar slow deamination of L-aristeromycin and related carbocyclic analogues, see ref 42. Thus, although the stereoselectivity of ADA is very high, it is not absolute.

(41) Bloch, A.; Leonard, R. J.; Nichol, C. A. *Biochim. Biophys. Acta* **1967**, *138*, 10.

(42) Secrist, J. A., III; Montgomery, J. A.; Shealy, Y. F.; O'Dell, C. A.; Clayton, S. J. *J. Med. Chem.* **1987**, *30*, 746.

(43) Lowe, G. *J. Chem. Soc., Chem. Commun.* **1965**, 411.

(44) Zemlicka, J. *Nucleosides Nucleotides* **1984**, *3*, 245.

(45) Kreis, W.; Watanabe, K. A.; Fox, J. J. *Helv. Chim. Acta* **1978**, *61*, 1011.

(46) Adenosine Deaminase in Disorders of Purine Metabolism and in Immune Deficiency (Tritsch, G. L., Ed.) *Ann. N.Y. Acad. Sci.* **1985**, *451*, 1–98.

(47) Vinogradov, S. N.; Linnell, R. H. *Hydrogen Bonding*; Van Nostrand Reinhold: New York, 1971; p 14.

(48) Maycock, A.; Abeles, R. *Acc. Chem. Res.* **1976**, *9*, 313.

(49) Lin, T.-S.; Schinazi, R. F.; Chen, M. S.; Kinney-Thomas, E.; Prusoff, W. H. *Biochem. Pharmacol.* **1987**, *36*, 311.

(50) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.* **1987**, *30*, 1270.

(51) Bennett, L. L., Jr.; Allan, P. W.; Hill, D. L. *Mol. Pharmacol.* **1968**, *4*, 208.

(52) Mitsuya, H.; Matsukura, M.; Broder, S. In *AIDS, Modern Concepts and Therapeutic Challenges*; Broder, S., Ed.; Marcel Dekker: New York, 1987; p 303.

(53) Hoshino, H.; Shimizu, N.; Shimada, N.; Takita, T.; Takeuchi, T. *J. Antibiot.* **1987**, *40*, 1077.

(54) Thus, acyclovir (**1b**) is able to potentiate[55] the anti-HIV activity of AZT, but it is not active per se whereas (*S*)-HPMPA is a nucleotide analogue effective[56] against Moloney mouse sarcoma retrovirus (MSV).

(55) Mitsuya, H.; Broder, S. *Nature* **1987**, *325*, 773.

(56) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464.

Experimental Section

General Methods. See ref 57. The following solvent systems were used for thin-layer chromatography (TLC): (S_1) dichloromethane–methanol (9:1); (S_2) dichloromethane–methanol (4:1); (S_3) tetrahydrofuran (THF)–methanol (9:1). Melting points were determined on a Reichert Thermovar apparatus, and they are uncorrected. The NMR spectra were measured in CD_3SOCD_3 unless stated otherwise. For the purpose of assignment of NMR signals, the prime numbering of substituents attached to nucleic acid bases is used (see formulas **3** and **8**). Electron-impact (EI-MS), chemical ionization (CI-MS), and fast atom bombardment mass spectra (FAB-MS) were determined with a Kratos MS80 RFA high-resolution instrument. High-performance liquid chromatography (HPLC) was run on an Altex Spectraphysics instrument with a Kratos Spectroflow 773 UV detector (at 254 nm) on a SynChropak RP-P column (SynChrom, Inc.) in 0.1 M KH_2PO_4 and 0.05 M NaCl (pH 4.5) as a buffer at a flow rate of 0.5 mL/min. K_2CO_3 was dried for 24 h at 100 °C before use. Optical rotations were determined with a Perkin-Elmer 141 polarimeter and circular dichroism (CD) spectra with a JASCO J-600 CD spectrometer. Adenosine deaminase (from calf intestine, ADA, EC 3.5.4.4) and purine-nucleoside phosphorylase (from bovine spleen, PNP, EC 2.4.2.1) were products of Sigma Chemical Co., St. Louis, MO). Cytidine deaminase (CDA, EC 3.5.4.5) was obtained from bovine kidney acetone powder as described.[58]

***N*^{9}-(4-Chloro-2-butyn-1-yl)adenine (5a).** A mixture of adenine (**4a**, 1.35 g, 10 mmol), K_2CO_3 (2.76 g, 20 mmol), and 1,4-dichloro-2-butyne (4.88 g, 40 mmol) in DMSO (50 mL) was stirred for 18 h at room temperature. The solution was evaporated, and the residue was washed several times with solvent system S_1 (total 300 mL). The crude product obtained by evaporation was chromatographed on a silica gel column in the same solvent. The major UV-absorbing fraction was evaporated to give compound **5a** (1.17 g, 53%), mp 199–201 °C after crystallization from ethyl acetate–methanol (95:5): UV (ethanol) max 260 nm (ϵ 15 600); ^{1}H NMR δ 8.18 and 8.15 (2 s, H_2 and H_8), 7.29 (s, 2, NH_2), 5.11 (t, 2, $H_{1'}$), 4.47 (t, 2, $H_{4'}$); EI-MS 221, 223 (M, 13.2, 3.9). Anal. Calcd for $C_9H_8ClN_5$: C, 48.76; H, 3.63; Cl, 15.99; N, 31.59. Found: C, 48.82; H, 3.82; Cl, 16.10; N, 31.48.

***N*^{1}-(4-Chloro-2-butyn-1-yl)cytosine (5c).** The procedure for **5a** was slightly modified for alkylation of cytosine (**4c**). The residue after evaporation of DMSO was washed with solvent system S_2 and the crude product was chromatographed in solvent S_1 to give compound **5c** (1 g, 51%), mp 189–191 °C after crystallization from ethyl acetate–methanol (4:1): UV (ethanol) max 272 nm (ϵ 8900), 206 (ϵ 16 000); ^{1}H NMR δ 7.61 (d, 1, H_6), 7.17 (d, 2, NH_2), 5.69 (d, 1, H_5), 4.54 and 4.45 (2 d, 4, $H_{1'}$ and $H_{4'}$); CI-MS 198, 200 (M + 1, 16.4, 5.9). Anal. Calcd for $C_8H_8ClN_3O$: C, 48.62; H, 4.08; Cl, 17.94; N, 21.26. Found: C, 48.71; H, 4.27; Cl, 18.18; N, 21.26.

***N*^{1}-(4-Chloro-2-butyn-1-yl)-5-methylcytosine (5d).** The procedure for **5a** was followed with some modifications, starting from 5-methylcytosine (**4d**). The reaction time was 3 days, the residue after evporation of DMSO was washed with CH_2Cl_2–methanol (3:2, 120 mL), and the crude product was chromatographed in solvent system S_1 to give compound **5d** (0.84 g, 40%), mp 171–174 °C after crystallization from THF–methanol (9:1): UV (ethanol) max 280 nm (ϵ 7400), 205 (ϵ 15 300), sh 238 (ϵ 7300); ^{1}H NMR δ 7.46 (s, 1 H_6), 7.32 and 6.79 (2 br s, 2, NH_2), 4.53 and 4.45 (2 t, 4, $H_{1'}$ and $H_{4'}$), 1.81 (s, 3, CH_3); CI-MS 212, 214 (M + 1, 100, 33.6). Anal. Calcd for $C_9H_{10}ClN_3O$: C, 51.07; H, 4.76; Cl, 16.75; N, 19.85. Found: C, 51.00; H, 4.96; Cl, 16.91; N, 19.96.

2-Amino-6-chloro-*N*^{9}-(4-chloro-2-butyn-1-yl)purine (5e). The procedure for compound **5a** was followed by use of 2-amino-6-chloropurine (**4e**). The crude product was chromatographed in CH_2Cl_2–ether (1:1) to give **5e** (1.2 g, 48%), mp 158–160 °C after crystallization from cyclohexane–ethyl acetate (4:1): UV (ethanol) max 309 nm (ϵ 7200), 221 (ϵ 21 000), 247 (ϵ 6400); ^{1}H NMR δ 8.17 (s, 1, H_8), 7.04 (s, 2, NH_2), 5.03 (t, 2, $H_{1'}$), 4.47 (t, 2, $H_{4'}$); EI-MS 255, 257 (M, 28.1, 18.0). Anal. Calcd for $C_9H_7Cl_2N_5$: C, 42.20; H, 2.75; Cl, 27.68; N, 27.34. Found: C, 42.16; H, 2.91; Cl, 27.45; N, 27.39.

***N*^{9}-(4-Hydroxy-2-butyn-1-yl)adenine (6a).** A solution of compound **5a** (1.1 g, 5 mmol) in 0.1 M HCl (50 mL) was refluxed for 18 h. After cooling, it was brought to pH 7 with NaOH and evaporated. The residue was chromatographed on a silica gel column in solvent system S_1. The major UV-absorbing fraction gave product **6a** (0.68 g, 68%), mp 221–223 °C after crystallization from 90% methanol: UV (pH 7) max 260 nm (ϵ 13 900), 208 (ϵ 20 200); ^{1}H NMR δ 8.16 (2 s, 2, H_2 + H_8), 7.21 (s, 2, NH_2), 5.16 (t, 1, OH), 5.03 (t, 2, $H_{1'}$), 4.07 (m, 2, $H_{4'}$); EI-MS 203 (M, 31.5), 202 (M – H, 100.0). Anal. Calcd for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.46. Found: C, 53.17; H, 4.40; N, 34.51.

***N*^{9}-(4-Hydroxy-2-butyn-1-yl)guanine (6b).** Compound **5e** was hydrolyzed as described for **6a**. The solids obtained after evaporation of neutralized aqueous solution were repeatedly washed with CH_2Cl_2–methanol (3:2, total 180 mL). The organic phase was evaporated, and the residue was chromatographed with ethyl acetate–methanol (3:2) to give **6b** (0.63 g, 58%), mp 257–258 °C after crystallization from 80% methanol: UV (pH 7) max 252 nm (ϵ 7200), 205 (ϵ 9400), sh 269 (ϵ 6100); ^{1}H NMR identical with that described;[17] FAB-MS 220 (M + H, 100.0). Anal. Calcd for $C_9H_9N_5O_2 \cdot H_2O$: C, 45.56; H, 4.67; N, 29.52. Found: C, 45.29; H, 4.29; N, 29.59.

***N*^{1}-(4-Hydroxy-2-butyn-1-yl)cytosine (6c).** The reaction with **5c** was performed as in the case of compound **6a**. Product **6c** was obtained after chromatography with solvent system S_2 as an eluent (0.54 g, 60%), mp 191–193 °C after crystallization from ethyl acetate–methanol (3:2): UV (pH 7) max 271 nm (ϵ 9500), 204 (ϵ 14 600); ^{1}H NMR δ 7.63 (d, 1, H_6), 7.17 (s, 2, NH_2), 5.70 (d, 1, H_5), 5.22 (t, 1, OH), 4.49 (t, 2, $H_{1'}$), 4.06 (t, 2, $H_{4'}$); EI-MS 179 (M, 19.1). Anal. Calcd for $C_8H_9N_3O_2$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.81; H, 5.15; N, 23.39.

***N*^{1}-(4-Hydroxy-2-butyn-1-yl)-5-methylcytosine (6d).** The procedure for compound **6a** was followed starting from intermediate **5d** on a 1-mmol scale to give **6d** (0.13 g, 68%), mp 201–204 °C after crystallization from THF–methanol (9:1): UV (pH 7) max 277 nm (ϵ 8500), 211 (ϵ 12 900); ^{1}H NMR δ 7.46 (s, 1, H_6), 7.25 and 6.75 (2 s, 2, NH_2), 5.19 (t, 1, OH), 4.48 (t, 2, $H_{1'}$), 4.06 (t, 2, $H_{4'}$); EI-MS 193 (M, 59.8). Anal. Calcd for $C_9H_{11}N_3O_2$: C, 55.94; H, 5.74; N, 21.74. Found: C, 55.82; H, 5.86; N, 21.64.

***N*^{2}-[(Dimethylamino)methylene]-*N*^{9}-(4-hydroxy-2-butyn-1-yl)guanine (6f).** A mixture of compound **6b** (1.09 g, 5 mmol), dimethylformamide dineopentyl acetal (1.15 g, 5 mmol), and DMF (30 mL) was stirred overnight at room temperature. The resultant solution was evaporated, and the residue was chromatographed on a silica gel column with solvent system S_2. The major UV-absorbing fraction was evaporated to give **6f** (0.82 g, 60%), mp 246–249 °C after crystallization from ethyl acetate–methanol (4:1): UV (pH 7) max 294 nm (ϵ 21 400), 232 (ϵ 14 400), 200 (ϵ 4300); ^{1}H NMR δ 11.29 (s, 1, NH), 8.57 [s, 1, =CH of (dimethylamino)methylene], 7.86 (s, 1, H_8), 5.22 (t, 1, OH), 4.93 (s, 1, $H_{1'}$), 4.09 (q, 1, $H_{4'}$), 3.14 and 3.01 (2 s, 6, CH_3). Anal. Calcd for $C_{12}H_{14}N_6O_2$: C, 52.54; H, 5.14; N, 30.64. Found: C, 52.33; H, 5.27; N, 30.49.

(±)-*N*^{9}-(4-Hydroxy-1,2-butadien-1-yl)adenine (3a, Adenallene). (A). Isomerization with 0.1 M NaOH at 100 °C. Compound **6a** (0.61 g, 3 mmol) was refluxed in 0.1 M NaOH (25 mL), and the progress of isomerization was followed by TLC in solvent S_1. After 30 min, the mixture contained ca. 50% of **3a**. The solution was cooled to 0–5 °C (ice bath); it was brought to pH 7 with 0.1 M HCl (pH meter) and evaporated. The residue was chromatographed on a silica gel column with solvent S_1. The fractions containing adenallene (**3a**) were combined and evaporated to give 0.2 g (33%) of **3a**, mp 189–190 °C after crystallization from ethyl acetate–methanol (9:1): UV (pH 7) max 260 nm (ϵ 13 900), 209 (ϵ 27 200); ^{1}H NMR δ 8.17 (2 s, 2, H_2 and H_8), 7.37 (m, 3, NH_2 and $H_{1'}$), 6.22 (q, 1, $H_{3'}$), 5.17 (t, 1, OH), 4.12 (m, 2, $H_{4'}$); ^{13}C NMR δ 195.64 ($C_{2'}$), 105.79 ($C_{3'}$), 93.78 ($C_{1'}$), 58.81 ($C_{4'}$), adenine peaks at 156.03, 152.94, 148.35, 138.35, and 118.87; EI-MS 203 (M, 73.4). Anal. Calcd for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.46. Found: C, 53.41; H, 4.44; N, 34.66.

(B) Isomerization with Potassium *tert*-Butoxide in DMF. A mixture of **6a** (203 mg, 1 mmol) and freshly sublimed potassium *tert*-butoxide (224 mg, 2 mmol) was stirred under N_2 in DMF (15 mL) for 1.5 h at −10 °C. Water (10 mL) was then added, the solvents were evaporated, and the residue was chromatographed as in method A to give adenallene (**3a**, 100 mg, 50%) identical (mp, TLC, and UV) with a sample prepared by method A.

(±)-*N*^{1}-(4-Hydroxy-1,2-butadien-1-yl)cytosine (3c, Cytallene). (A) Isomerization with 0.1 M NaOH at 100 °C. Compound **6c** (716 mg, 4 mmol) was refluxed in 0.1 M NaOH in 20% aqueous dioxane (30 mL) for 9 h. The progress of isomerization was monitored by TLC (S_3, developed three to four times). The mixture was then worked up as described for adenallene (**3a**, method A). Chromatography in solvent system S_1 gave compound **3c** (200 mg, 28%), homogeneous on TLC (S_3, triple development). This material was crystallized three times from methanol to give **3c**, mp 186–190 °C, 94–97% pure according to ^{1}H NMR: UV (pH 7) max 290 nm (ϵ 11 300), 224 (ϵ 12 000), 204 (ϵ 13 900); IR (KBr) 1965 cm^{-1} (C=C=C); ^{1}H NMR δ 7.50 (d, 1, H_6), 7.34 and 7.27 (m, 3, NH_2 + $H_{1'}$), 6.12 (q, 1, $H_{3'}$), 5.80 (d, 1, H_5), 5.05 (t, 1, OH), 4.03 (m, 2, $H_{4'}$); ^{13}C NMR δ 193.90 ($C_{2'}$), 106.83 ($C_{3'}$), 99.32 ($C_{1'}$), 59.08 ($C_{4'}$), cytosine peaks at 165.44, 153.64, 140.86, and 95.47; CI-MS 180 (M + H, 80.7). Anal. Calcd for $C_8H_9N_3O_2$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.42; H, 5.13; N, 23.33.

(57) Ben Cheikh, A.; Craine, L. E.; Recher, S. G.; Zemlicka, J. *J. Org. Chem.* **1988**, 53, 929.
(58) Liu, P. S.; Marquez, V. E.; Driscoll, J. S.; Fuller, R. W.; McCormack, J. *J. Med. Chem.* **1981**, *24*, 662.

(B) See Adenallene (3a, Method B). A mixture of compound **6c** (268 mg, 1.5 mmol) and potassium *tert*-butoxide (336 mg, 3 mmol) was stirred for 14 h at room temperature under N_2. The progress of isomerization was monitored by TLC as in method A. The mixture was worked up in the usual fashion. Chromatography afforded compound **3c** (214 mg, 80%), mp 173–180 °C. This product was crystallized to a constant mp of 193–194 °C (four times) from methanol to give **3c** (39%), 99% pure according to ^{1}H NMR and identical with a sample prepared by method A.

(±)-N^1-(4-Hydroxy-1,2-butadien-1-yl)-5-methylcytosine (3d). Procedure B for allene **3c** was employed on a 1-mmol scale with acetylene **6d** and an equimolar amount of potassium *tert*-butoxide; progress of the reaction was checked by TLC (S_1). To an ice-cold solution, 1% aqueous acetic acid (5 mL) was added, and the mixture was evaporated. Chromatography (S_3) afforded allene **3d** in two portions, pure **3d** (48 mg, 25%), mp 185–189 °C after crystallization from THF–methanol (4:1), and 63 mg (32%) contaminated with acetylene **6d**: UV (pH 7) max 297 nm (ε 8800), 224 (ε 9800); ^{1}H NMR δ 7.65 and 6.97 (2 s, 2, NH_2), 7.28 (m, 2, H_6 and $H_{1'}$), 6.10 (q, 1, $H_{3'}$), 5.06 (t, 1, OH), 4.05 (m, 2, $H_{4'}$), 1.85 (s, 3, CH_3); ^{13}C NMR δ 193.82 ($C_{2'}$), 106.78 ($C_{3'}$), 99.13 ($C_{1'}$), 59.20 ($C_{4'}$), 13.06 (CH_3), cytosine peaks at 165.32, 153.68, 137.70, 102.95. Anal. Calcd for $C_9H_{11}N_3O_2$: C, 55.94; H, 5.74; N, 21.74. Found: C, 55.79; H, 6.00; N, 21.53.

(±)-N^2-[(Dimethylamino)methylene]-N^9-(4-hydroxy-1,2-butadien-1-yl)guanine (3f). The reaction was performed as in the case of allene **3a** (method B) on a 2-mmol scale with **6f** and an equimolar amount of potassium *tert*-butoxide in DMF (25 mL) at 80 °C (bath temperature) for 5 h. After the reaction was cooled with ice, 1% aqueous acetic acid (10 mL) was added, and the mixture was evaporated. The residue was flash chromatographed in solvent system S_2 to give 0.27 g (50%) of compound **3f** containing according to ^{1}H NMR 80% allene and 20% acetylene, mp 230 °C dec after crystallization from ethyl acetate–methanol (4:1). Further crystallizations did not raise the mp but decreased the allene/acetylene ratio: UV (pH 7) max 297 nm (ε 21 300), 231 (ε 16 500), 206 (ε 17 600); ^{1}H NMR δ 11.36 (br s, 1, NH), 8.63 [s, 1, =CH of (dimethylamino)methylene], 7.84 (s, 1, H_8), 7.28 (m, 1, $H_{1'}$), 6.16 (q, 1, $H_{3'}$), 5.15 (t, 1, OH), 4.11 (m, 1, $H_{4'}$), 3.14 and 3.02 (2 s, 6, CH_3).

(±)-N^9-(4-Hydroxy-1,2-butadien-1-yl)guanine (3b, Guanallene). Compound **3f** (0.27 g, 1 mmol) was stirred in methanol saturated with NH_3 (0 °C) for 16 h at room temperature. The solution was evaporated, and the residue was chromatographed on a silica gel column in solvent system S_2. The major UV-absorbing fraction was evaporated to give allene **3b** (0.12 g, 55%), mp 190–210 °C. Attempted crystallization resulted in partial decomposition and a decrease of the allene/acetylene ratio. This product was homogeneous on TLC (S_2), and it contained ca. 90% allene **3b** (^{1}H NMR): UV (pH 7) max 256 nm (ε 7000), 206 (ε 12 000), sh 270 (ε 6200); ^{1}H NMR δ 10.77 (br s, 1, NH), 7.72 (s, 1, H_8), 7.07 (m, 1, $H_{1'}$), 6.63 (s, 2, NH_2), 6.18 (q, 1, $H_{3'}$), 5.13 (t, 1, OH), 4.09 (m, 1, $H_{4'}$). Anal. Calcd for $C_9H_9N_5O_2$·4 H_2O: C, 37.11, H, 5.88; N, 24.04. Found: C, 36.89, H, 5.50; N, 24.32.

(±)-N^6-[(Dimethylamino)methylene]adenallene (3g). A mixture of adenallene (**3a**, 0.2 g, 1 mmol) and dimethylformamide dimethyl acetal (0.48 g, 4 mmol) was stirred in DMF (15 mL) for 16 h at room temperature. The solution was evaporated, and the residue was chromatographed on a silica gel column in CH_2Cl_2–methanol (97:3) to give compound **3g** (0.23 g, 91%), mp 151–153 °C after crystallization from ethyl acetate. UV (pH 7) max 312 (ε 31 700), 229 (ε 22 300); ^{1}H NMR ($CDCl_3$) δ 8.98 [s, 1, =CH of (dimethylamino)methylene], 8.55 and 7.97 (2s, 2, H_2 and H_8), 7.20 (m, 1, $H_{1'}$), 6.20 (m, 1, $H_{3'}$), 4.43 (m, 2, $H_{4'}$), 3.29 and 3.23 (2 s, 6, CH_3). Anal. Calcd for $C_{12}H_{14}N_6O$: C, 55.79; H, 5.46; N, 32.54. Found: C, 55.82; H, 5.59; N, 32.58.

(±)-N^9-(4-Hydroxy-1,2-butadien-1-yl)hypoxanthine (3h, Hypoxallene): Complete Deamination of (±)-Adenallene (3a). A mixture of adenallene (**3a**, 100 mg, 0.5 mmol) and adenosine deaminase (30 mg) was stirred in 0.05 M Na_2HPO_4 (15 mL, pH 7.5) at room temperature for 14 h. The solution was evaporated, and the residue was extracted with boiling solvent S_2 (three times with 25 mL). The organic phase was evaporated, and the residue was chromatographed on a silica gel column in solvent S_1. The major UV-absorbing fraction was evaporated to give hypoxallene (**3h**) (90 mg, 90%), mp 210 °C after crystallization from ethyl acetate–methanol (4:1) mixture: UV (pH 7) max 222 nm (ε 26 500), 208 (ε 25 900), sh 254 (ε 17 800); ^{1}H NMR δ 12.40 (br s, 1, NH), 8.10 (2 s, 2, H_2 and H_8), 7.34 (m, 1, $H_{1'}$), 6.22 (q, 1, $H_{3'}$), 5.05 (br s, 1, OH), 4.12 (m, 2, $H_{4'}$); ^{13}C NMR δ 195.85 ($C_{2'}$), 106.14 ($C_{3'}$), 93.80 ($C_{1'}$), 58.74 ($C_{4'}$), hypoxanthine peaks at 156.44, 147.15, 146.21, 137.90, and 124.36. Anal. Calcd for $C_9H_8N_4O_2$: C, 52.93; H, 3.94; N, 27.44. Found: C, 52.62; H, 4.12; N, 27.27.

(−)-Adenallene (3a) and (+)-Hypoxallene (3h): Incomplete Deamination of (±)-Adenallene (3a). A mixture of adenallene (**3a**, 20 mg, 0.1 mmol) and adenosine deaminase (4 mg) in 0.05 M Na_2HPO_4 (pH 7.5, 5 mL) was stirred at room temperature for 40 min. The mixture contained a ca. 1:1 ratio of **3h** and **3a** according to TLC (S_1). The solution was evaporated, and the residue was chromatographed on a silica gel column with solvent S_1, which eluted (−)-adenallene (**3a**, 9 mg, 45%): $[\alpha]^{25}_D$ −83.3° (*c* 0.9, methanol); CD (pH 7) max 234 nm ([θ] −19 400). Elution with S_2 gave (+)-hypoxallene (**3h**, 6 mg, 30%): $[\alpha]^{25}_D$ 18.3° (*c* 0.6, methanol); CD (pH 7) max 232 nm ([θ] 16 100).

N^9-(4-Hydroxybut-1-yl)adenine (2a). Adenallene (**3a**, 50 mg, 0.25 mmol) was hydrogenated in ethanol (30 mL, Parr apparatus) with 10% Pd/C (50 mg) for 15 h at 50 psi. The catalyst was filtered off (Celite pad), and the filtrate was evporated to give compound **2a** (48 mg, 95%), mp 196–199 °C after crystallization from ethyl acetate–methanol (95:5): lit.[19] mp 196–197 °C; UV (pH 7) max 260 nm (ε 13 300), 209 (ε 15 600).

(±)-N^9-(4-Chloro-1,2-butadien-1-yl)adenine (7a). A mixture of adenallene (**3a**, 0.61 g, 3 mmol), triphenylphosphine (1.79 g, 4.5 mmol), and CCl_4 (1.5 mL, 15 mmol) was stirred in DMF (20 mL) for 15 h at room temperature. The solvent was evaporated, and the residue was chromatographed on a silica gel column in CH_2Cl_2–methanol (97:3) to give compound **7a** (0.62 g, 93%), mp 175 °C dec after crystallization from THF–cyclohexane (4:1): UV (ethanol) max 258 nm (ε 13 000), 216 (ε 16 200); IR (KBr) 1961 cm^{-1} (C=C=C); ^{1}H NMR[59] δ 8.17 (s, 2, H_2 plus H_8), 7.60 (m, 1, $H_{1'}$), 7.39 (s, 2, NH_2), 6.38 (q, 1, $H_{3'}$), 4.37 (d, 2, $H_{4'}$); ^{13}C NMR δ 197.91 ($C_{2'}$), 101.81 ($C_{3'}$), 94.57 ($C_{1'}$), 41.67 ($C_{4'}$), adenine peaks at 155.95, 152.97, 148.40, 138.40, and 118.87; EI-MS 221, 223 (M, 3.1, 1.1). Anal. Calcd for $C_9H_8N_5Cl$: C, 48.76; H, 3.63; Cl, 15.99; N, 31.60. Found: C, 49.00; H, 3.64; Cl, 16.13; N, 31.70.

(±)-N^9-(1-Oxa-3-cyclopenten-2-yl)adenine (8a). Butyne **6a** (0.41 g, 2 mmol) was refluxed in 1 M NaOH in 50% dioxane (20 mL) for 1 h. The progress of the reaction was followed by TLC (S_2). Prolonged heating led to decomposition. After cooling (5–10 °C), the solution was brought to pH 7 with 0.1 M HCl (pH meter) whereupon it was evaporated. The residue was extracted several times with solvent S_2 (total 150 mL). The insoluble portion was filtered off, the filtrate was evaporated, and the crude product was chromatographed on a silica gel column in CH_2Cl_2–methanol (95:5) to give 62 mg (17%) of **8a**, mp 148–151 °C, and adenallene (**3a**, 0.12 g, 27%): UV (pH 7) max 259 nm (ε 12 800), 210 (ε 15 400); ^{1}H NMR δ 8.15 and 8.03 (2 s, 2, H_2 plus H_8), 7.28 (s, 2, NH_2), 6.95 (sept, 1, $H_{2'}$), 6.57 (d of q, 1, $H_{3'}$), 6.09 (m, 1, $H_{4'}$), 4.87 and 4.65 (d of oct and d of q, 2, $H_{5'}$); ^{13}C NMR δ 133.45 and 123.80 ($C_{3'}$ plus $C_{4'}$), 88.36 ($C_{2'}$), 75.25 ($C_{5'}$), adenine peaks at 156.01, 152.79, 149.07, 138.66, and 118.99; EI-MS 203 (M, 0.3), 69 ($C_4H_5O^+$, 12.8), 68 (furan, 32.2), 39 ($C_3H_3^+$, 41.1), adenine peaks at 135, 108, 81, and 54; CI-MS 204 (M + H, 22.9), 136 (**4a** + H, 100.0). Anal. Calcd for $C_9H_9N_5O \cdot ^1/_{16}H_2O$: C, 52.90; H, 4.50; N, 34.28. Found: C, 53.19; H, 4.63; N, 34.00.

(±)-N^9-(1-Oxa-3-cyclopenten-2-yl)guanine (8b). The reaction was performed as described for compound **8a** on a 5-mmol scale of butyne **6b**. The progress of the reaction was followed in a THF–methanol (4:1 or 3:2) solvent system. The cooled solution was neutralized with Dowex 50 (H^+), the resin was filtered off, it was washed with water (total 40 mL), and the filtrate was lyophilized. The residue was mixed with silica gel (1 g), and it was applied on a column made of the same material, which was eluted with THF–methanol (3:2) to give compound **8b** (0.19 g, 27%), mp 190 °C dec: UV (pH 7) max 250 nm (ε 7800), sh 270 (ε 6000); ^{1}H NMR δ 10.95 (s, 1, NH), 7.51 (s, 1, H_8), 6.78 (s, 2, NH_2), 6.69 (m, 1, $H_{2'}$), 6.54 (m, 1, $H_{3'}$), 6.04 (m, 1, $H_{4'}$), 4.81 and 4.62 (2 m, 2, $H_{5'}$); ^{13}C NMR δ 133.45 and 123.87 ($C_{3'}$ + $C_{4'}$), 87.82 ($C_{2'}$), 75.02 ($C_{5'}$), guanine peaks at 156.79, 154.12, 150.76, 134.73, and 116.71. Anal. Calcd for $C_9H_9N_5O_2 \cdot 4H_2O$: C, 37.11; H, 5.88; N, 24.04. Found: C, 37.16; H, 5.63; N, 24.02.

(±)-N^1-(1-Oxa-3-cyclopenten-2-yl)cytosine (8c). The reaction with butyne **6c** was performed on a 1-mmol scale as described for compound **8a** to give **8c** (68 mg, 38%), mp 161–163 °C after crystallization from ethyl acetate: UV (pH 7) max 269 (ε 8800); ^{1}H NMR δ 7.24 (d, 1, H_6), 7.23 (s, 2, NH_2), 6.88 (sept, 1, $H_{2'}$), 6.48 (d of q, 1, $H_{3'}$), 5.86 (decet, 1, $H_{4'}$), 5.71 (d, 1, H_5), 4.77 and 4.57 (d of oct and d of q, 2, $H_{5'}$); ^{13}C NMR δ 132.81 and 125.13 ($C_{3'}$ + $C_{4'}$), 90.41 ($C_{2'}$), 75.05 ($C_{5'}$), cytosine peaks at 165.64, 155.23, 140.89, and 94.53; EI-MS 179 (M, 5.8), 69 ($C_4H_5O^+$, 43.6), 68 (furan, 100.0), 39 ($C_3H_3^+$, 98.8), cytosine peaks at 111, 95, and 83. Anal. Calcd for $C_8H_9N_3O_2$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.48; H, 5.27; N, 23.26.

(±)-N^9-(1-Oxacyclopentan-2-yl)adenine (12a). Oxacyclopentene **8a** (40 mg, 0.2 mmol) was hydrogenated as described for compound **2a** to give **12a**, mp 161–163 °C after crystallization from petroleum ether–benzene (9:1), 36 mg (90%), and mp 162–164 °C after two additional

(59) The sample was dissolved in CD_3SOCD_3 immediately before the measurement; otherwise, an extra set of peaks was obtained owing to the reaction of **7a** with the solvent.

recrystallizations, lit.[25] mp 163–165 °C, undepressed on admixture with an authentic sample: UV (pH 7) max 260 nm (ε 13 100), 206 (ε 16 400).

Deamination of 3a and 6a with ADA. Substrate **3a** or **6a** (2.2–2.4 μmol) was incubated with ADA (0.4 unit, 0.2 unit/mol) in 0.05 M Na_2HPO_4 (pH 7.5, 0.4 mL) at room temperature. Aliquots were periodically withdrawn, and they were examined by TLC (S_1) and UV spectroscopy. The deamination was quantitative in both cases after 6 h.

Deamination of L-Adenosine with ADA. L-Adenosine (2 μmol) was incubated with ADA as described above in 0.05 M K_2HPO_4. The withdrawn aliquots were examined by paper chromatography in 2-propanol–NH_4OH–water (7:1:2) on Whatman 3MM paper and by UV spectrophotometry of the excised spots of L-adenosine and L-inosine. The deamination was 50% complete after 6 days and quantitative after 34 days. Control experiments without the enzyme (L-adenosine) and with ADA (tubercidin) showed that both compounds were stable after 8 days of incubation. D-Adenosine was quantitatively deaminated at 43 μM concentration in 4 min.

Stability of Cytallene (3c) toward CDA. Compound **3c** (2.2 μmol) was incubated with CDA (2×10^{-3} unit) in 0.05 M Na_2HPO_4 (pH 7, 0.4 mL) for 40 h at room temperature. Aliquots which were periodically withdrawn and examined by TLC (S_1) showed only the presence of unchanged **3c**. In a control experiment (pH 8, 8×10^{-4} unit of CDA/μmol of substrate) cytidine (0.2 mM) was deaminated at a rate of 7×10^{-3} OD_{280} unit/min as estimated spectrophotometrically.

Stability of Hypoxallene (3h) toward PNP. Compound **3h** (2.4 μmol) was incubated with PNP (0.4 unit) for 24 h in 0.05 M Na_2HPO_4 (pH 7.5, 0.4 mL). TLC (S_1) and UV indicated no reaction after 24 h and then with 4.4 units of enzyme after an additional 24 h. By contrast, guanosine (0.19 mM) was converted quantitatively to guanine within 20 min (1.6 units of PNP/μmol of substrate) as shown by UV spectrophotometry at 253 nm.[60]

Acknowledgment. Thanks are due to the Central Instrumentation Facility (Department of Chemistry, Wayne State University, Dr. Robin Hood, Director) for NMR and mass spectral data. We are grateful to Dr. W. B. Butler (MCF) for computer assistance, to Dr. Barry Schoenfelner (CIF) for his help in obtaining the CD spectra, and to Dr. J. Pitha, National Institute of Aging, NIH, Baltimore, MD, for a sample of N^9-vinyladenine. L-Adenosine was obtained through courtesy of Dr. H. B. Wood, National Cancer Institute, NIH, Bethesda, MD. The work described herein was supported in part by U.S. Public Health Service Research Grant CA 32779 from the National Cancer Institute and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. The CD spectrometer was obtained with the aid of BRS Shared Instrumentation Grant S10 03366 from the Division of Research Resources, NIH, Bethesda, MD.

(60) **Note Added in Proof**: After submission of this paper, syntheses of compounds **6a** and **6b** by different methods were published: (a) Borcherding, D. R.; Narayanan, S.; Hasobe, M.; McKee, J. G.; Keller, B. T.; Borchardt, R. T. *J. Med. Chem.* **1988**, *31*, 1729. (b) Ashton, W.; Canning Meurer, L.; Cantone, C. L.; Field, A. K.; Hannah, J.; Karkas, J. D.; Liou, R.; Patel, G. F.; Perry, H. C.; Wagner, A. F.; Walton, E.; Tolman, R. L. *J. Med. Chem.* **1988**, *31*, 2304. Also, another inhibitor of HIV lacking the oxacyclopentane moiety (1′a-carba-2′,3′-didehydro-2′,3′-dideoxyguanosine, carbovir) was reported: Vince, R.; Hua, M.; Brownell, J.; Daluge, S.; Lee, F.; Shannon, W. M.; Lavelle, G. C.; Qualls, J.; Weislow, O. S.; Kiser, R.; Canonico, P. G.; Schultz, R. H.; Narayanan, V. L.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1046.

Biosynthesis of Antibiotics of the Virginiamycin Family. 8.[1] Formation of the Dehydroproline Residue

Michael B. Purvis,[2] Joseph W. LeFevre,[2] Vickie L. Jones,[2] David G. I. Kingston,*[,2] André M. Biot,[3] and Francis Gosselé[3]

Contribution from the Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212, and Smith Kline—RIT, B-1330 Rixensart, Belgium. *Received July 20, 1988*

Abstract: The formation of the dehydroproline residue of the antibiotic virginiamycin M_1 occurs with equal facility from both (*R*)- and (*S*)-proline; the 3-*pro-R* proton of proline is lost stereospecifically in this process. *cis*-3-Hydroxyproline but not *trans*-3-hydroxyproline is incorporated into the antibiotic, although less efficiently than proline, and virginiamycin M_2 is converted into virginiamycin M_1. These results suggest that virginiamycin M_1 is most probably formed by incorporation of (*S*)-proline into virginiamycin M_2, which then undergoes hydroxylation with retention of configuration and elimination of water to yield virginiamycin M_1.

The antibiotics of the virginiamycin family contain a rich diversity of unusual and interesting amino acids. Antibiotics of group B, such as virginiamycin S_1, contain 3-hydroxypicolinic acid, which may be viewed as a dehydrolysine.[1] Antibiotics of group A, such as virginiamycin M_1 (VM_1, **1**), contain both a dehydroproline unit and an oxazole ring;[4] the latter may be viewed as a dehydroserine moiety.

The origin of α,β-dehyro amino acids in natural products has been the subject of considerable speculation, and it has variously been suggested that they arise by tautomerization of an acyl imino intermediate[5] or by dehydration of hydroxy amino acids.[6,7] The relationship between (*R*)-amino acids and α,β-dehydro amino acids has also been discussed, and the suggestion that (*R*)-amino acids arise from dehydro amino acids[5] has been refuted.[8] In this

(1) Paper 7: Reed, J. W.; Kingston, D. G. I.; Purvis, M. B.; Biot, A.; Gosselé, F. *J. Org. Chem.* **1989**, *54*, 1161–1165.
(2) Department of Chemistry, Virginia Polytechnic Institute and State University.
(3) Smith Kline-Recherche et Industrie Thêrapeutiques.
(4) Delpierre, G. R.; Eastwood, F. W.; Gream, G. E.; Kingston, D. G. I.; Todd, A. R.; Williams, D. H. *J. Chem. Soc. C* **1966**, 1653–1669. Kingston, D. G. I.; Todd, A. R.; Williams, D. H. *J. Chem Soc. C* **1966**, 1669–1676.
(5) Bycroft, B. W. *Nature (London)* **1969**, *224*, 595–597.
(6) Pearce, C. J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1979**, *101*, 5069–5070. Rinehart, K. L., Jr.; Weller, D. D.; Pearce, C. J. *J. Nat. Prod.* **1980**, *43*, 1–20.
(7) Schmidt, U.; Häusler, J.; Ohler, E.; Poisel, H. *Fortschr. Chem. Org. Naturst.* **1979**, *37*, 251–327.

