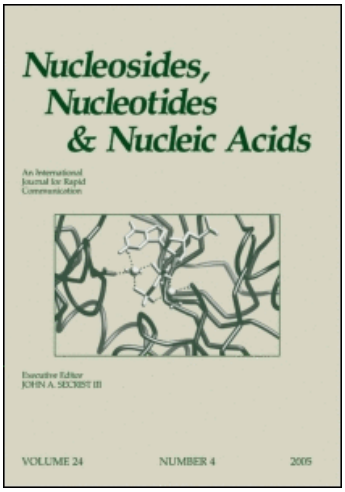

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Antiviral Nucleoside Analogues with Axial Chirality

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ANTIVIRAL NUCLEOSIDE ANALOGUES WITH AXIAL CHIRALITY

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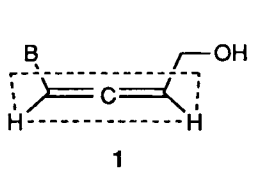
ABSTRACT. Chemistry and biological activity of nucleoside analogues comprising a chiral axis will be reviewed with emphasis on anti-HIV agent adenallene (**1a**) and anti-HIV/HBV analogue cytallene (**1b**). Possible mechanism of action, activity toward the enzymes of nucleic acid metabolism and structure-activity relationships will be discussed.

In the past several years we have developed a new class of biologically active nucleoside analogues comprising a system of cumulated double bonds[1] (**1**). These compounds were designed as analogues of 2',3'-dideoxyribonucleosides (**2**) which exhibit a strong antiretroviral activity[2]. Formally, the tetrahydrofuran moiety of 2',3'-dideoxyribo-



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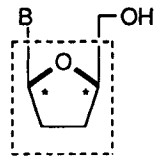

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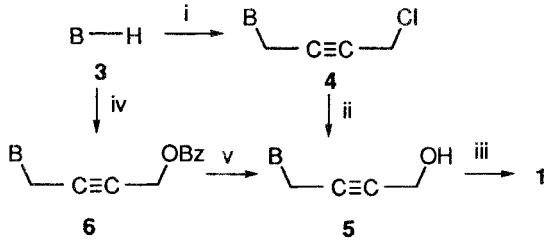
1a, 2a: B = Ade
1b, 2b: B = Cyt
1c, 2c: B = Hyp

nucleoside possessing two chirality centers was replaced by an allene grouping with a chiral axis. Modeling studies indicated that this change did not appreciably influence the distance between the nucleic acid base and hydroxymethyl group. Both functions are essential for biological activity of 2',3'-dideoxyribonucleosides. Not surprisingly, there are some noticeable differences in stereochemistry of both types of analogues. In 2',3'-dideoxyribonucleosides the rotation of the base and hydroxymethyl groups is restricted but the ribofuranose (tetrahydrofuran) ring maintains a degree of flexibility. On the other hand, the allene moiety of **1** is more rigid than tetrahydrofuran ring but the rotation of the base and hydroxymethyl function is free. Thus, in rough terms, it is a trade-off between mobility and rigidity in both groups of analogues.

Biological results provided additional and the most rewarding aspect of similarity between both types of analogues (Table 1). The extent of inhibition of cytopathic effect and

TABLE 1. Inhibition of cytopathic effect and replication of HIV-1 in ATH8 cells by adenallene (**1a**), cytallene (**1b**), 2',3'-dideoxyadenosine (ddA, **2a**) and 2',3'-dideoxycytidine (ddC, **2b**)[3].

	Adenallene (**1a**)	Cytallene (**1b**)	ddA (**2a**)	ddC (**2b**)
EC_{50} (μM)	14	0.1	6	0.2
CC_{50} (μM)	>200	>5	>600	20



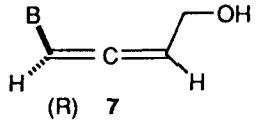
i. $ClCH_2C{\equiv}CCH_2Cl$, K_2CO_3, DMSO.
ii. 0.1 M HCl, Δ.
iii. tBuOK, DMF.
iv. $BrCH_2C{\equiv}CCH_2OBz$, K_2CO_3, DMF.
v. NH_3, MeOH.
Series a: B = Ade; series b: B = Cyt

Scheme 1

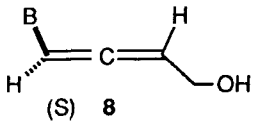
replication of HIV-1 in ATH8 cells caused by adenallene (**1a**) and cytallene (**1b**) closely paralleled that of 2',3'-dideoxyadenosine (**2a**) and 2',3'-dideoxycytidine[3] (**2b**). These results were encouraging considering the fact that they are biased in favor of optically pure 2',3'-dideoxyribonucleosides whereas both allene derivatives were tested as racemic mixtures.

The synthesis of allene analogues **1** makes use of readily obtainable acetylenic precursors (Scheme 1). Thus, adenine (**3a**) was alkylated with 1,4-dichlorobutyne and the resultant intermediate **4a** was subjected to acid hydrolysis to give the corresponding butynol **5a**. Alternately, alkylation with 1-benzoyloxy-4-bromo-2-butyne afforded the intermediary benzoate **6a** which was then debenzoylated to give butynol[4] **5a**. Isomerization of the latter effected by a strong base led to an equilibrium mixture of allene **1a** and acetylene **5a** containing approximately 80 % of **1a**. Pure allene **1a** was obtained by column chromatography and crystallization. In the case of cytallene (**1b**), the whole process (**3b** -> **6b** -> **5b** -> **1b**) was accomplished without a single chromatographic separation on a large scale[5] (40 g of **3b**) in 25 % overall yield.

Allenes **1** are racemic mixtures of R and S enantiomers (**7** and **8**). Because of a strong anti-HIV activity of adenallene (**1a**) and cytallene (**1b**) it was of obvious interest to investigate enantioselectivity of antiretroviral effect and determine absolute configuration



(R) **7**



(S) **8**

7a, 8a: B = Ade
7b, 8b: B = Cyt
8c: B = Hyp

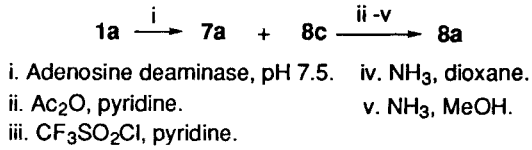
of active enantiomers. For this purpose, we needed optically pure enantiomers of both analogues.

On an analytical scale, adenallene[6] (**1a**) and cytallene[5] (**1b**) were resolved using chiral HPLC columns. Interestingly, column suitable for resolution of adenallene (**1a**) /cellulose triacetate, Chiralcel CA-1/ was totally ineffective for cytallene (**1b**). Conversely, cytallene (**1b**) was resolved on a column of tribenzoyl cellulose (Chiralcel OB) which did not resolve adenallene (**1a**). This method was, of course, poorly suitable for a preparative separation of enantiomers of **1a** and **1b** but it provided a valuable tool for assessing the efficiency of other methods of resolution.

Adenallene (**1a**) is a substrate of moderate activity for adenosine deaminase from calf intestine. When the incubation is carried out for a prolonged period of time (24 h) racemic adenallene (**1a**) is completely converted to racemic hypoxallene (**1c**). When the deamination was stopped at about 50 % conversion optically active (-)-adenallene (**7a**) and (+)-hypoxallene (**8c**) were obtained[6] (Scheme 2). It is convenient to follow the reaction course of deamination by chiral HPLC to determine the conditions for highest possible optical yield of **7a**. By this method, (-)-adenallene (**7a**) was obtained in >95 % optical purity. The absolute configuration of **7a** is R as determined by X-ray diffraction[6].

For a side-by-side comparison of anti-HIV activity of both enantiomers of adenallene it was necessary to convert the S-(+)-hypoxallene (**8c**) to S-(+)-adenallene (**8a**). Again, conventional procedures were applied for this purpose[6] (Scheme 2). The S-(+)-hypoxallene (**8c**) was first acetylated to give the respective acetate which was then activated with trifluoromethylsulfonyl anhydride and pyridine followed by ammonolysis. Finally, deacetylation with ammonia in methanol provided optically pure S-(+)-adenallene (**8a**).

The HIV-assays in ATH8 host cells established[6] that R-(-)-adenallene (**8a**) is the biologically active enantiomer (Table 2). The S(+)-enantiomer (**8a**) was virtually inactive. It should be emphasized that the R-(-)-enantiomer (**7a**) is a direct product of a controlled deamination of racemic compound with adenosine deaminase (Scheme 2). This is fortuitous but very fortunate and of practical significance because the biologically active enantiomer can be obtained directly and in a single step from the racemic mixture. It is also clear that R-enantiomer (**7a**) with a high anti-HIV activity is the least readily deaminated. We can then conclude that both enantiomers of adenallene **7a** and **8a** can mimic a nucleoside but with a different enantioselectivity for different receptors (enzymes). A similar relationship was noted in case of AIDS drug 3TC (lamivudine) where the enantiomer with highest antiviral activity was the most resistant toward cytidine deaminase[7,8].



Scheme 2

TABLE 2. Inhibition of cytopathic effect and replication of HIV-1 in ATH8 cells by R-adenallene (**7a**) and S-adenallene (**8a**)[6].

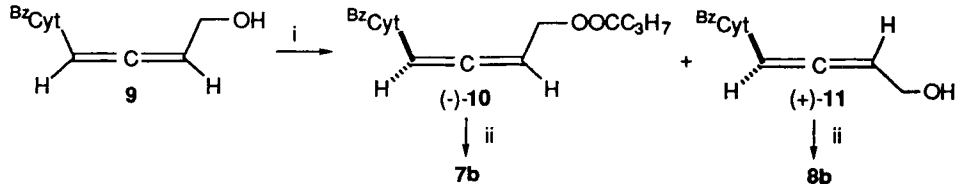
	R-Adenallene (**7a**)	S-Adenallene (**8a**)
EC_{50} (μM)	5.8	>200
CC_{50} (μM)	>200	>200

An approach similar to that used for resolution of racemic adenallene (**1a**) was precluded given the fact that cytallene (**1b**) is not a substrate for cytidine deaminase. Therefore, we turned our attention to lipase-catalyzed acylations in organic solvents[9]. Such an approach would offer an additional advantage of keeping the heterocyclic base intact during the process of resolution.

It was possible that introduction of a lipophilic substituent into the molecule of cytallene (**1b**) could provide an increased binding affinity and selectivity for the large lipophilic binding pocket of a lipase. We have therefore prepared N^4-benzoylcytallene (**9**) for this purpose.

Investigation of acylation kinetics of N^4-benzoylcytallene (**9**) with vinyl butyrate and lipase AK in tetrahydrofuran (THF) reinforced the original assumption that the benzoyl group may improve enantioselectivity of the reaction. Therefore, acylation catalyzed by lipase AK was used for a preparative resolution[5] (Scheme 3). Thus, N^4-benzoylcytallene (**9**) was acylated with vinyl butyrate and lipase AK in THF. Molecular sieves 4A were also included to scavenge acetaldehyde formed during the esterification. The (-)-ester **10** and unreacted (+)-enantiomer **11** were separated by column chromatography and they were deacylated by ammonia in methanol. The chiral HPLC indicated that optical purity of both (-)- and (+)-cytallene **7b** and **8b** was at least 94 %.

As in the case of adenallene, the absolute configuration of (+)-enantiomer **8b** determined by X-ray diffraction[5] is S. The (+)-adenallene (**8a**) has also an S configuration[6].



i. $C_3H_7CO_2CH{=}CH_2$, lipase AK, molecular sieves 4A, THF. ii. NH_3, MeOH.

Scheme 3

In contrast to nucleosides[10], the change of heterocyclic moiety has apparently little effect on the sign of optical rotation.

Anti-HIV activity of R- and S-cytallene **7b** and **8b** was determined in phytohemagglutinin-stimulated peripheral blood mononuclear (PHA-PBM) cells infected with a primary HIV-1 isolate[5] (Table 3). For comparison, racemic cytallene (**1b**) was also tested. Again, it was the R-enantiomer **7b** which was the most active. The S-enantiomer **8b** was virtually inactive. As expected, R-enantiomer **7b** was twice as potent than the racemic analogue **1b**.

In addition to anti-HIV activity, cytallene (**1b**) is also a strong inhibitor of replication of hepatitis B virus[11] (HBV). Again, R-enantiomer **7b** is the active form twice as potent than racemic cytallene (**1b**, Table 3). Both R-enantiomer **7b** and racemic analogue **1b** are eight times more effective against HBV than against HIV. The R-cytallene (**7b**) is not only significantly more potent against HBV than 2',3'-dideoxycytidine (**2b**) but it exhibits a much lower long-term (mitochondrial) toxicity (CC_{50} > 10 μM), an important parameter for further drug development. By contrast, adenallene (**1a**) was totally inactive.

There are significant differences in the mechanism of action of adenallene (**1a**) and cytallene (**1b**)[1]. Both analogues inhibit HIV-1 and HIV-2 and, therefore, phosphorylated intermediates are involved in their metabolism. Deoxycytidine kinase is capable of phosphorylating cytallene[12] (**1b**) but enzyme(s) responsible or phosphorylation of adenallene (**1a**) have not been identified. More recent experiments with prodrug of adenallene **12** indicated that phosphorylated forms are indeed involved in the mechanism[13] of action (Scheme 4). Analogue **12** which exhibits anti-HIV activity significantly higher than adenallene (**1a**) is probably converted by intracellular esterase(s) to phosphamidate **13** which, in turn, yields the monophosphate **14**. However, the mechanism of transformation of **13** to **14** remains unclear.

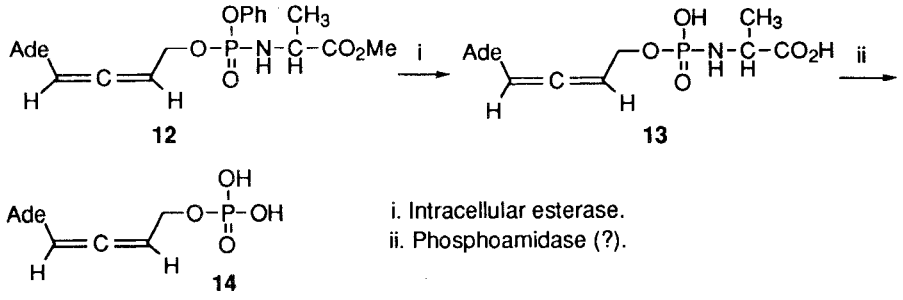
It was already mentioned that adenosine deaminase showed a distinct preference for deamination of S-adenallene (**8a**, Scheme 2). By contrast, racemic adenallene 4'-phos-

TABLE 3. Anti-HIV and Anti-HBV Activities of Racemic Cytallene (**1b**), R-enantiomer **7b** and S-enantiomer **8b**.

Analogue	HIV-1[a]		HBV[b]	
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
(±)-Cytallene (**1b**)	0.8	>10	0.1	80
R-Cytallene (**7b**)	0.4	>10	0.05	10
S-Cytallene (**8b**)	>10	>10	Inactive	

[a]Phytohemagglutinin-stimulated peripheral blood mononuclear (PHA-PBM) cells infected with a primary HIV-1 isolate[5].

[b]HBV-transfected cell line 2.2.15. Cytotoxicity was determined in cultured CEM cells[11].



Scheme 4

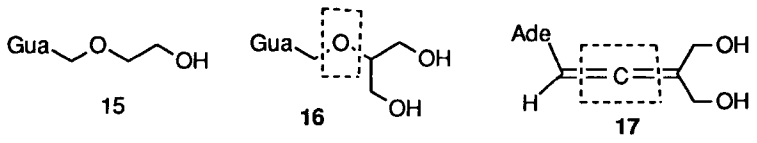
phate (**14**) was deaminated[14] by AMP deaminase with no apparent enantioselectivity. Compound **14** is a poor substrate for 5'-nucleotidase. However, there is a significant preference for hydrolysis of the R-phosphate. It is then interesting to note that the enantioselectivity is opposite to adenosine deaminase-catalyzed deamination and it corresponds to that observed for enzyme-catalyzed acylations[5] of adenallene (**1a**) and cytallene (**1b**). It should be also noted that deamination of both enantiomers is a major metabolic route of adenallene (**1a**) in murine leukemia L1210 cells[15].

The studies of structure - activity relationships in the limited series of allenic analogues indicated that adenallene (**1a**) and cytallene (**1b**) are the only analogues possessing a high anti-HIV activity[1]. The guanine analogue (guanallene), hypoxallene (**1c**), 2,6-diamino-

purine or thymine analogue (thymallene) were devoid of antiretroviral activity in contrast to the respective 2',3'-dideoxyribonucleoside analogues[1,3]. These results point to some limitations of analogy to 2',3'-dideoxyribonucleosides. This is clearly apparent in the case of hypoxallene (**1c**) which was totally devoid of anti-HIV activity although 2',3-dideoxyinosine (**2c**, ddI) is a drug for treatment of AIDS. The most likely explanation of this difference is a more limited capability of intracellular phosphorylation of allenic analogues relative to 2',3'-dideoxyribonucleosides.

By contrast, 5-fluorocytallene showed an antiretroviral activity[16] although significantly less potent than 5-fluoro-2',3'-dideoxycytidine. The antiviral activity of 5-fluorocytallene in HIV-1 infected ATH8 cells was only borderline with EC_{50} 100 μM whereas the cytotoxicity was not observed up to 500 μM. Nevertheless, in PHA-PBM cells infected with a clinical isolate of the virus, the anti-HIV effect was significantly greater (EC_{50} 3.8 μM).

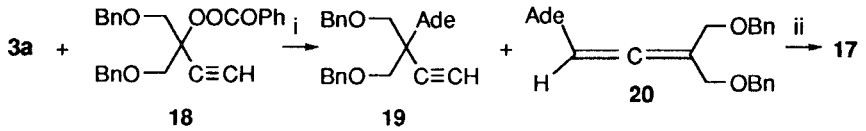
The rationale shown in formulas **1** and **2** can be extended by considering adenallene (**1a**) as an analogue of acyclovir (**15**), an acyclic nucleoside and important drug against herpes virus infections. A hydroxymethyl derivative of **15**, ganciclovir (**16**), is also a drug used for treatment of cytomegalovirus afflictions. It was then of interest to examine the corresponding 3'-hydroxymethyl derivative of adenallene **17** whose sp (allenic) carbon is isoelectronic with ether oxygen of ganciclovir (**16**).



15 16 17

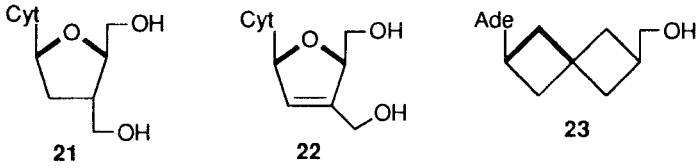
The synthetic approach to 3'-hydroxymethyladenallene (**17**) was based on alkylation of adenine with a suitable precursor of the allene moiety[17] (Scheme 5). 1,3-Dibenzyloxyacetone was transformed to acetylenic carbinol which was *in situ* converted to phenyl carbonate **18**. Alkylation of adenine (**3a**) with **18** gave acetylene **19** and allene **20** in low yields (8-9 %). Deprotection of **20** with boron trichloride in dichloromethane afforded 3'-hydroxymethyladenallene (**17**). Attempts to apply this approach to the synthesis of 3'-hydroxymethylallenes of other nucleobases were not successful. The 3'-hydroxymethyladenallene (**17**) is a substrate for adenosine deaminase but it is devoid of any antiviral activity.

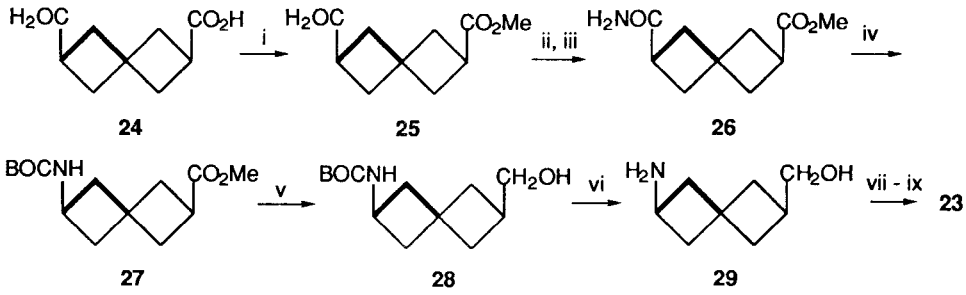
It is interesting to note that attachment of an additional potential binding function (e. g., hydroxymethyl group) to rigid unsaturated systems can significantly lower the anti-HIV effect. Thus, 3'-hydroxymethyl analogue **21** is a potent anti-HIV agent[18] whereas the corresponding unsaturated derivative **22** is inactive[19]. Apparently, some additional flexibility of the analogue is required to accommodate such a group at the appropriate receptor binding site.



Scheme 5

The SAR studies have indicated that the presence of cumulated double bonds is indispensable for antiretroviral activity of adenallene (**1a**) and cytallene (**1b**). Nevertheless, it must be stressed that in all non-allenic models studied the original axial chirality of the allene system was destroyed[1]. The exact role of the allene grouping remains to be elucidated. It is not clear whether it serves only as a spacer between the heterocyclic bases and hydroxymethyl group or whether it also participates in the binding with receptor, e. g., as an acceptor of hydrogen bond. We have therefore examined another model compound, a spirocyclic analogue of adenallene **23**. In this analogue, the axial chirality is conserved





i. $Me_2CH(OMe)_2$, DMF.
ii. iBuOCOCl, NEt_3, THF.
iii. NH_3, THF.
iv. $Pb(OAc)_4$, tBuOH, DMF.
v. $CaBH_4$, THF.
vi. HCl, MeOH.
vii. 5-Amino-4,6-dichloropyrimidine, NEt_3, BuOH, Δ.
viii. $CH(OEt)_3$, TsOH, DMF.
ix. NH_3, MeOH, Δ.

Scheme 6

but no unsaturated linkages are present. Also, the distance between the heterocyclic base and hydroxymethyl group is increased relative to adenallene (**1a**).

Synthesis of analogue **23** started from Fecht's acid (**24**) which was esterified using dimethylformamide dimethyl acetal to give monoester[20] **25** (Scheme 6). Activation with isobutyl chloroformate followed by reaction with ammonia afforded amide ester **26**. The Hoffman rearangement and trapping the isocyanate intermediate with tert.-butyl alcohol gave the protected aminoester **27**. Reduction with calcium borohydride led to the primary alcohol **28**. Deprotection with hydrochloric acid in methanol furnished aminoalcohol **29** as a hydrochloride. The adenine ring was constructed in a conventional fashion which included the following steps: (1) Reaction with 5-amino-4,6-dichloropyrimidine; (2) purine ring closure with triethyl orthoformate and p-toluenesulfonic acid; (3) ammonolysis.

The spirocyclic analogue **23** is resistant to adenosine deaminase. It is not an active anti-HIV agent but it inhibited the human cytomegalovirus (HCMV) in human foreskin fibroblast (HFF) culture with IC_{50} 32 μM and it had about the same level of activity against murine leukemia L1210. This is a moderate activity but analogue **23** is racemic and the efficacy of the active enantiomer should be higher.

Acknowledgment. Many excellent scientists have participated in this multidisciplinary effort. Their contribution is reflected in publications that resulted from the project. A generous financial support from the National Cancer Institute, Bethesda, Maryland (grant CA32779) and the United Way of Southeastern Michigan is also acknowledged.

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