(*R*)-(−)- and (*S*)-(+)-Synadenol: Synthesis, Absolute Configuration, and Enantioselectivity of Antiviral Effect[⊗]

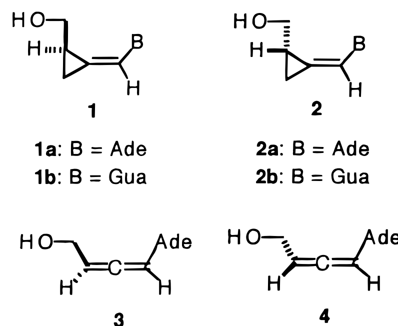
Yao-Ling Qiu,[†] Andrew Hempel,[‡] Norman Camerman,[‡] Arthur Camerman,[§] Fiona Geiser,[∥] Roger G. Ptak,[▽] Julie M. Breitenbach,[▽] Toshiko Kira,[⊥] Ling Li,[⊥] Elizabeth Gullen,[⊥] Yung-Chi Cheng,[⊥] John C. Drach,[▽] and Jiri Zemlicka*,[†]

Department of Chemistry, Experimental and Clinical Chemotherapy Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201-1379, Biochemistry Department, University of Toronto, Toronto, Ontario M5S 1A8, Canada, Ardono Research, Seattle, Washington 98105, Chiral Technologies, Inc., Exton, Pennsylvania 19341, Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510-8066, and Department of Biologic and Materials Science, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48019-1078

Received May 26, 1998

Synthesis of (*R*)-(−)- and (*S*)-(+)-synadenol (**1a** and **2a**, 95−96% ee) is described. Racemic synadenol (**1a** + **2a**) was deaminated with adenosine deaminase to give (*R*)-(−)-synadenol (**1a**) and (*S*)-(+)-hypoxanthine derivative **5**. Acetylation of the latter compound gave acetate **6**. Reaction with *N*,*N*-dimethylchloromethyleneammonium chloride led to 6-chloropurine derivative **7**. Ammonolysis furnished (*S*)-(+)-synadenol (**2a**). Absolute configuration of **1a** was established by two methods: (i) synthesis from (*R*)-methylenecyclopropanecarboxylic acid (**8**) and (ii) X-ray diffraction of a single crystal of (−)-synadenol hydrochloride. Racemic methylenecyclopropanecarboxylic acid (**10**) was resolved by a modification of the described procedure. The *R*-enantiomer **8** was converted to ethyl ester **13** which was brominated to give vicinal dibromides **14**. Reduction with diisobutylaluminum hydride then furnished alcohol **15** which was acetylated to the corresponding acetate **16**. Alkylation−elimination procedure of adenine with **16** yielded acetates **17** and **18**. Deprotection with ammonia afforded a mixture of *Z*- and *E*-isomers **1a** and **19** of the *R*-configuration. Comparison with products **1a** and **2a** by chiral HPLC established the *R*-configuration of (−)-synadenol (**1a**). These results were confirmed by X-ray diffraction of a single crystal of (−)-synadenol hydrochloride. The latter forms a pseudosymmetric dimer with adenine−adenine base pairing in the lattice with the nucleobase in an *anti*-like conformation. Enantiomers **1a** and **2a** exhibit varied enantioselectivity toward different viruses. Both enantiomers are equipotent against human cytomegalovirus (HCMV) and varicella zoster virus (VZV). The *S*-enantiomer **2a** is somewhat more effective than *R*-enantiomer **1a** in herpes simplex virus 1 and 2 (HSV-1 and HSV-2) assays. By contrast, enantioselectivity of antiviral effect is reversed in Epstein-Barr virus (EBV) and human immunodeficiency virus type 1 (HIV-1) assays where the *R*-enantiomer **1a** is preferred. In these assays, the *S*-enantiomer **2a** is less effective (EBV) or devoid of activity (HIV-1).

In our previous reports[1−4] we described a new series of nucleoside analogues comprising a methylenecyclopropane function and exhibiting a strong antiviral effect against a broad range of viruses. Of this group of analogues, synadenol (**1a** + **2a**) and synguanol (**1b** + **2b**) are of particular importance because of their inhibition of replication of human and murine cytomegalovirus (HCMV and MCMV) and Epstein-Barr virus (EBV) as well as human herpes virus 6 (HHV-6). Synadenol (**1a** + **2a**) is also an agent against hepatitis B virus (HBV) and varicella zoster virus (VZV). Compounds **1a** + **2a** and **1b** + **2b** as well as related analogues which contain a single chiral center were obtained by chemical synthesis as racemic mixtures.[1−3] Resolution of the racemates or synthesis of enantiopure biologically active enantiomers is then a foremost task in further development of these analogues as potential antiviral drugs. Because methylenecyclopropane nucleoside mimics were originally designed as analogues of the corresponding allenes,[1] a comparison of enantioselectivity of antiviral effects in both series of analogues is also of interest. In this communication, we report on synthesis, absolute configuration, and antiviral activity of (*R*)- and (*S*)-synadenol (**1a** and **2a**).

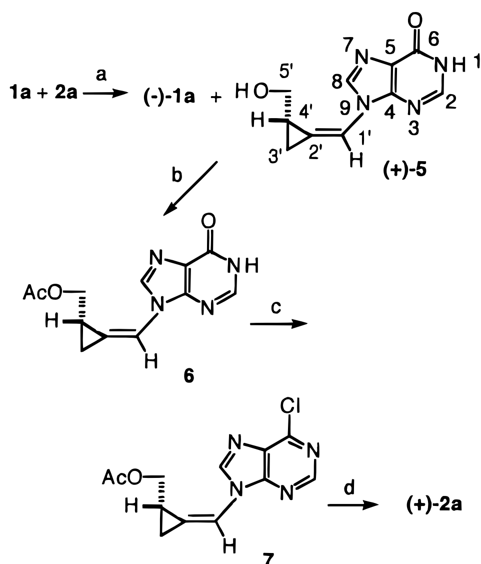


[⊗] Presented in part at the 13th International Roundtable: Nucleosides, Nucleotides and Their Biological Applications, Montpellier, France, Sept 6−10, 1998; Abstracts Poster 16; *Nucleosides Nucleotides*, in press.

* Send correspondence to this author at the Barbara Ann Karmanos Cancer Institute, 110 E. Warren Ave., Detroit, MI 48201-1379. Telephone: (313) 833-0715, ext. 2452 or 2262. Fax: (313) 832-7294. E-mail: zemlicka@kci.wayne.edu.
[†] Wayne State University.
[‡] University of Toronto.
[§] Ardono Research.
[∥] Chiral Technologies, Inc.
[▽] University of Michigan.
[⊥] Yale University.

10.1021/jm980323u CCC: $15.00 © 1998 American Chemical Society
Published on Web 12/01/1998

Scheme 1[a]



[a] (a) Adenosine deaminase, pH 7.5; (b) Ac_2O, pyridine; (c) $[Me_2N{=}CHCl]^+Cl^-$, $CHCl_3$, △; (d) NH_3, MeOH, △.

Synthesis

Previously,[1] in a preliminary experiment, we found that synadenol (**1a** + **2a**) is a substrate for adenosine deaminase from calf intestine. The reaction kinetics indicated that after 48 h the deamination stopped at approximately 50% conversion. This result suggested that digestion with adenosine deaminase could possibly be utilized for resolution of enantiomers. A similar approach was used before in our laboratory[5] for resolution of the anti-HIV agent adenallene (**3** + **4**). In a preparative experiment, deamination of (±)-synadenol (**1a** + **2a**) afforded the (−)-enantiomer **1a** in 39% yield, whereas the deaminated product, (+)-hypoxanthine derivative **5**, was obtained in 44−48% yield (Scheme 1). Compound **5** was converted to (+)-synadenol (**2a**) as follows. Acetylation furnished the acetoxy derivative **6** in almost quantitative yield. Chlorination by *N,N*-dimethylchloromethyleneammonium chloride[6] in refluxing $CHCl_3$ gave 6-chloropurine derivative **7** (88%). Ammonolysis with NH_3 in methanol at 100 °C in an autoclave afforded (+)-synadenol (**2a**) in 96% yield. It is interesting to note that similar conditions proved destructive for an analogous allene derivative[5] indicating a higher stability of the methylenecyclopropane system. The optical purity of enantiomers **1a** (95% ee) and **2a** (96% ee) was determined by chiral HLPC on a Chiralpak AD column using methanol as an eluent (Figure 1, panels A−C).

Absolute Configuration

Synadenol (**1a** + **2a**) and adenallene (**3** + **4**) have the same type of chromophore (a double bond attached to the adenine base), and the center of dissymmetry either is a part of the chromophore (allene, **3** and **4**) or is located next to it (methylenecyclopropane, **1a** and **2a**). In fact, their UV spectra are almost superimposable. It seemed then possible that enantiomers of the same sign of optical rotation may have the same absolute configuration. Some support for such a reasoning is in the fact that a correlation between the absolute configuration of optically active allenes and methylenecyclopropanes

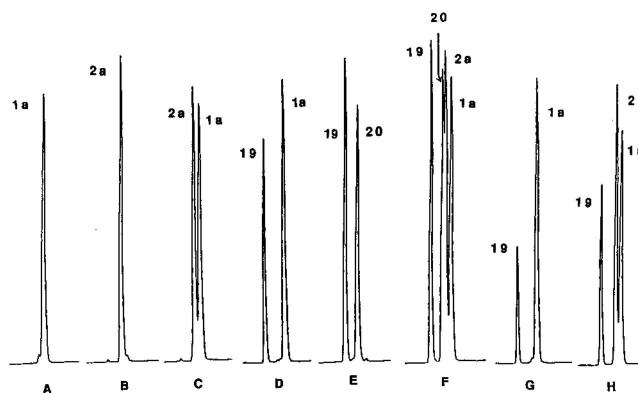


Figure 1. Chiral HPLC of enantiomers **1a**, **2a**, **19**, and **20**. Chiralpak AD column (10 μm, 4.6 × 250 mm, MeOH as eluent, flow rate 1 mL/min, detection at 261 nm, attenuation 16). The 20-μL aliquots of the following solutions in MeOH were injected: panel A, (*R*)-(−)-synadenol (**1a**) from deamination, 0.25 mg/mL; panel B, (*S*)-(+)-synadenol (**2a**), 0.25 mg/mL; panel C, (±)-synadenol (**1a** + **2a**), 0.5 mg/mL; panel D, (*R*)-synadenol (**1a**) and *R,E*-isomer **19** (1.5:1), 0.5 mg/mL; panel E, (±)-*E*-isomers **19** + **20**, 0.5 mg/mL; panel F, (±)-synadenol (**1a** + **2a**) and the (±)-*E*-isomers **19** + **20**, 1 mg/mL; panel G, 1:1 mixture of **1a** + **19** (panel D) and **1a** (panel A); panel H, 1:1 mixture of **1a** + **19** (panel D) and **2a** (panel B, attenuation 8). The retention times (min, panel F) for each enantiomer were as follows: 8.45 (**19**), 9.91 (**20**), 10.25 (**2a**), and 10.99 (**1a**).

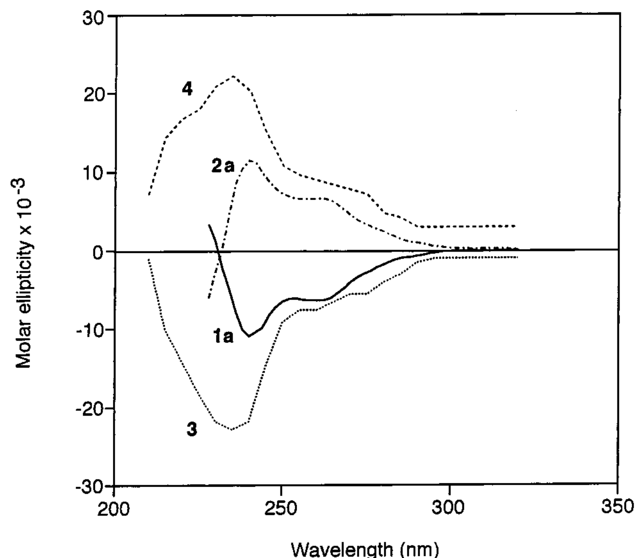
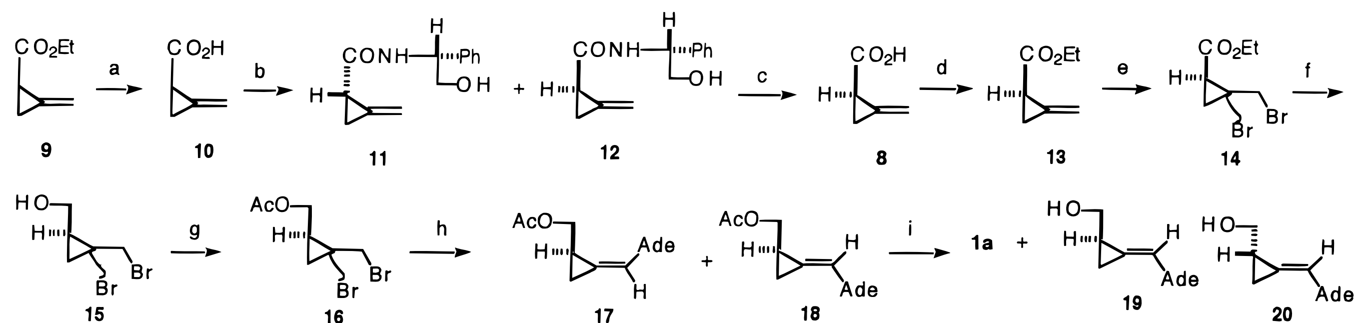


Figure 2. CD spectra of enantiomers of synadenol (**1a** + **2a**) and adenallene (**3** + **4**). For details see Experimental Section: (—) (*R*)-(−)-synadenol (**1a**); (· · ·) (*R*)-(−)-adenallene (**3**); (− · − · − ·) (*S*)-(+)-synadenol (**2a**); (− − −) (*S*)-(+)-adenallene (**4**).

was observed[7] although only in a limited number of cases. The values of optical rotations of 1,3-disubstituted methylenecyclopropane enantiomers are smaller than those of the corresponding allenes. This was also observed in our case because an absolute value of optical rotation $[\alpha]_D$ of (−)- and (+)-synadenol (**1a** and **2a**) is 120−123°, whereas that of (*R*)-(−)- and (*S*)-(+)-adenallene[5] amounts to 179−181°. Similarity of CD spectra of (*R*)-(−)- and (*S*)-(+)-adenallene (**3** and **4**) with (−)- and (+)-synadenol (**1a** and **2a**) (Figure 2) is also in line with the supposition that enantiomers with the same sign of Cotton effect may have the same absolute configuration. Enantiomers **1a**, **2a**, **3**, and **4** display the Cotton effects between 230 and 240 nm and a less-well-

Scheme 2[a]



[a] (a) KOH, MeOH/H_2O; (b) 1. iBuOCOCl, NEt_3, (*R*)-2-phenylglycinol, 2. separation; (c) 1 M H_2SO_4, THF; (d) HCl, EtOH; (e) Br_2, CCl_4; (f) DIBALH, THF; (g) Ac_2O, pyridine; (h) adenine, K_2CO_3, DMF, △; (i) NH_3/MeOH.

developed maximum at 260 nm (λ_{max} in the UV spectrum). It is noteworthy that molar ellipticity at 230−240 nm is significantly higher in enantiomers of adenallene **3** and **4** than synadenol **1a** and **2a**. This may be related to the fact that the chromophore is directly a part of the chiral axis in allenes **3** and **4**, whereas in the case of methylenecyclopropanes **1a** and **2a** it is located next to the chiral center.

Further support for a tentative assignment of *R*-configuration to the (−)-enantiomer **1a** comes from the biological data. We have previously shown[5] that (*S*)-(+)-adenallene is deaminated more readily than the (*R*)-(−)-enantiomer but the enantioselectivity of their anti-HIV effect is just the opposite. Because the same trend of substrate activity toward adenosine deaminase and anti-HIV potency was observed with (−)- and (+)-synadenol (**1a** and **2a**) (Scheme 1; for HIV data see Table 1), it seemed quite plausible that (−)-enantiomer **1a** has an *R*-configuration and (+)-enantiomer **2a** an *S*-configuration. Nevertheless, Cheng et al.[8] reached very recently an opposite conclusion which was based on calculated conformational parameters of a series of nucleoside analogues, and they assigned an *S*-configuration to (−)-synadenol (**1a**). These authors also resolved racemic synadenol (**1a** + **2a**) on a chiral column, but enantiomers **1a** and **2a** were characterized only by optical rotations which are significantly lower ($[\alpha]_D$ ±26.3°) than those described herein ($[\alpha]_D$ ±120−123°).

The absolute configuration of (−)- and (+)-synadenol (**1a** and **2a**) was determined by two methods: (A) correlation of (−)-synadenol (**1a**) with (*R*)-methylenecyclopropanecarboxylic acid[9] (**8**) and (B) single-crystal X-ray diffraction of hydrochloride of **1a**.

A. Correlation of (−)-Synadenol (1a) with (*R*)-Methylenecyclopropanecarboxylic Acid (8). The known[10] ethyl methylenecyclopropanecarboxylate (**9**) was hydrolyzed with KOH in aqueous methanol to give the corresponding racemic acid **10** in 98% yield (Scheme 2). The reported procedure[9] using K_2CO_3 in aqueous methanol proved unsuitable for this purpose. Compound **10** was then converted to the *R*,*S*- and *R*,*R*-diastereoisomeric amides of (*R*)-phenylglycinol **11** and **12** which were then resolved by a column chromatography on silica gel by a modification of the described procedure[9] in 39% and 41% yield, respectively. Acid hydrolysis of the diastereoisomer **12** gave quantitatively (*R*)-methylenecyclopropanecarboxylic acid[9] (**8**) which was readily esterified to afford ester **13**. Adddition of bromine provided 1*S*,2*S*- and 1*S*,2*R*-diastereoisomers **14**. A similar reaction of racemic **9** was recently described.[3,8] Compounds **14** could have been successfully used for an alkylation−elimination procedure with adenine.[1] However, to avoid any potential racemization of the chiral center next to the carboxylate function, the latter was reduced with diisobutylaluminum hydride (DIBALH) to give hydroxymethyl derivatives **15** in 75% overall yield based on four steps from amide **12**. This procedure was used previously[3] in the synthesis of racemic synadenol (**1a** + **2a**). Acetylation then furnished acetates **16** (96%). The racemic counterparts of **16** were recently described.[3] Alkylation−elimination procedure with adenine[3] afforded a mixture of acetates **17** and **18** in 86% yield. Deprotection with methanolic ammonia giave (*R*)-synadenol (**1a**) and the corresponding *E*-isomer **19** in quantitative yield, and the ratio of **1a**:**19** was 1.5:1 as determined by chiral HPLC (Figure 1, panel D). No racemization of **1a** and **19** was observed.

Enantiomers **1a**, **2a**, **19**, and **20** of the racemic *E*/*Z*-isomeric mixture[1] were all resolved on a Chiralpak AD column (Figure 1, panels C, E, and F) which permitted the determination of absolute configuraiton of (−)- and (+)-synadenol (**1a** and **2a**) obtained from enzymic deamination (Scheme 1) by a comparison with **1a** without separation of the *Z*- and *E*-isomers **1a** and **19**. Thus, Figure 1 (panel G) shows that addition of (−)-synadenol (**1a**) obtained from enzymic deamination of racemate **1a** + **2a** to a mixture of *Z*- and *E*-isomers **1a** and **19** of the *R*-configuration obtained by enantioselective synthesis from (*R*)-methylenecyclopropanecarboxylic acid (**8**) (Scheme 2) caused only an increase of the peak corresponding to the *R*-enantiomer **1a** as compared with panel D (Figure 1). By contrast, addition of (+)-synadenol (**2a**) resulted in a clear separation of the three peaks of **1a**, **2a**, and **19** (Figure 1, panel H). Therefore, the *R*-configuration must be assigned to the (−)-enantiomer **1a**. It is also worthy of note that alkylation agent **16** and the corresponding diastereoisomers derived from (*S*)-methylenecyclopropanecarboxylic acid[9] can be used for the synthesis of enantiopure (*R*)- and (*S*)-methylenecyclopropane analogues of nucleobases other than adenine by the procedures described for the corresponding racemic compounds.[1−3]

The configuration of (*R*)-methylenecyclopropanecarboxylic acid (**8**) was previously determined[9] by HPLC elution profiles of diastereoisomeric (*R*)-phenylglycinol derivatives **11** and **12** which were compared with patterns of similar amides derived from structurally unrelated compounds. In addition, optical rotations of

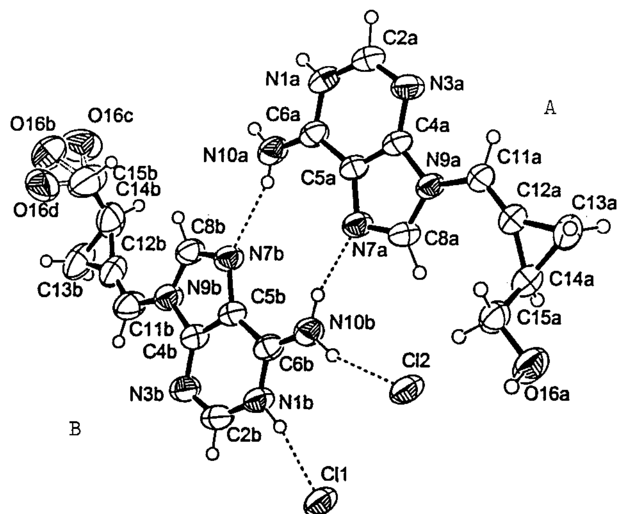


Figure 3. ORTEP-3 view of the two independent (−)-synadnol hydrochloride molecules showing 50% probability displacement ellipsoids and hydrogen-bonded A and B molecules forming dimers.

8, **11**, **12**, and the *S*-enantiomer of **8** were not reported. Therefore, the configuration of (−)-synadenol (**1a**) was independently confirmed by X-ray diffraction of a single crystal.

B. Crystallographic Results. The absolute configuration was determined by refinement of the Flack parameter for (−)-synadenol hydrochloride, which showed that (−)-synadenol (**1a**) has the *R*-absolute configuration according to Cahn, Ingold, and Prelog notation.[11] The two independent molecules of (−)-synadenol hydrochloride which constitute the crystallographic asymmetric unit are presented in Figure 3. Formation of the hydrochloride has occurred by protonation at N1 of adenine, the position also favored in adenine nucleosides.[12] The hydroxyl group of the B molecule is disordered; there are three partial occupancy positions for the O16 oxygen atom (occupancies refined to 0.48, 0.24, and 0.33 for O16b,c,d, respectively), and consequently two hydrogen atoms, one at O16 and one at C15, could not be reliably positioned from the difference map. Apart from this, the geometrical parameters (bonds and angles) of both molecules are in good agreement with commonly accepted values. Structural details are available as Supporting Information. The conformations of the two crystallographically independent molecules differ in the side chain. The cyclopropyl ring of the A molecule is coplanar with the plane of the adenine rings system plus N10 and C11, as opposed to the B molecule where the angle between normals to these planes is 12.9(6)°. In the crystal, the molecules are stacked along the *b*-axis direction and bound together by a three-dimensional hydrogen bond network. The two crystallographically independent molecules are paired by two symmetrical hydrogen bonds between N7 and the amino N10 group of the adenines; the dimers are bonded to other dimers through interactions involving N1, N10, and the chloride ions and through hydroxy groups (Table 2).

The X-ray diffraction studies also showed that the conformation of the base (adenine) is *anti* in both the hydrochloride of **1a** (Figure 3) and the free base (Supporting Information, Figure 1). The same conformation in solution (DMSO) was also indicated by the NMR spectra[1] of the racemic compound **1a** + **2a**. The adenine−adenine dimer displaying an unusual base pairing observed in the lattice of the hydrochloride of **1a** is of interest. A dimeric structure involving a different set of hydrogen bonds was also observed in crystals of (*R*)-(−)-adenallene[5] (**3**).

Our results have unambiguously shown that a recent assignment[8] of the *S*-configuration of (−)-synadenol (**1a**) is in error. The present investigation also provides conclusive support for the original configurational assignment of (*R*)-methylenecyclopropanecarboxylic acid[9] (**8**).

Antiviral Activity

Antiviral activity of (*R*)-(−)- and (*S*)-(+)-synadenol (**1a** and **2a**) was investigated with seven different viruses: human cytomegalovirus (HCMV), herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV), human immunodeficiency virus 1 (HIV-1), and hepatitis B virus (HBV) (Table 1). Most surprisingly, both enantiomers were virtually equipotent as inhibitors of replication of HCMV virus in plaque and yield reduction assay (EC_{50} 2.4−2.9 and EC_{90} 0.5−2.0 μM, respectively). The same phenomenon was observed in VZV-infected cell culture (EC_{50} 1.5 μM for both enantiomers). In HBV assay the *S*-enantiomer **2a** was somewhat more active (EC_{50} 9 μM) than the *R*-enantiomer **1a** (EC_{50} 15.7 μM). (±)-Synadenol (**1a** + **2a**) is only a moderate anti-HSV agent,[1] but some enantioselectivity was observed. Thus, the (*S*)-(+)-enantiomer **2a** with EC_{50} 8.8 (ELISA), 16, and 35 μM (Vero) is a somewhat better inhibitor of replication of both HSV-1 and HSV-2 than the (*R*)-(−)-enantiomer **1a** (EC_{50} 38 (ELISA) and >50 μM in Vero cell culture). In EBV/H-1 assay the *R*-enantiomer **1a** was more potent (EC_{50} 0.09 μM) than *S*-enantiomer **2a** (EC_{50} 0.62 μM). However, it is clear that both enantiomers are effective inhibitors of EBV. Addition of coformycin, an inhibitor of adenosine and AMP deaminase, did not influence the anti-EBV potency of **1a** and **2a**. This result indicated that neither of the enantiomers is subject to intracellular enzymic deamination. The (*S*)-(+)-synhypoxanthol (**5**) was inactive. Although (±)-synadenol (**1a** + **2a**) is only a moderate[1] inhibitor of HIV-1, the (*R*)-(−)-enantiomer **1a** showed an EC_{50} value of 11 μM, whereas the (*S*)-(+)-enantiomer **2a** was inactive. A similar pattern of activity was found in adenallene (**3** + **4**) which is a more potent anti-HIV agent than synadenol (**1a** + **2a**), where the (*R*)-(−)-enantiomer **3** is significantly more effective than the (*S*)-(+)-enantiomer[5] **4**.

The tested viruses can then be divided into three groups according to their response toward enantiomers **1a** and **2a**: (i) no enantioselectivity (HCMV, VZV), (ii) moderate enantioselectivity favoring the (*S*)-(+)-enantiomer **2a** (HSV-1, HSV-2, HBV), and (iii) enantioselective preference for the (*R*)-(−)-enantiomer **1a** (EBV, HIV-1).

Many antiviral nucleoside analogues are essentially prodrugs which have to be converted to effective agents (triphosphates) inhibiting the respective viral DNA polymerase or reverse transcriptase. Multiple enzymes (nucleoside and nucleotide kinases, nucleotidases, etc.) involved in the activation process (phosphorylation) can

Table 1. Antiviral Activity of (±)-Synadenol (**1a** + **2a**), *R*-(−)-Enantiomer **1a**, and *S*-(+)-Enantiomer **2a**

	antiviral activity, 50% inhibitory concentration (EC_{50}, μM)[a]							
		HSV-1						
compd	HCMV HFF	BSC-1[b]	Vero	HSV-2 Vero	EBV H-1	HIV-1 CEM-SS	HBV 2.215	VZV HEL
1a + **2a**	2.1[c] (1.3)[d]	26	28	59	0.2	17[e]	17	1.5
1a[f]	2.9[c] (2.0)[d]	38	>50	>50	0.09[g]	11[e]	15.7	1.5
2a[f]	2.4[c] (0.5)[d]	8.8	16	35	0.63[h,i]	>100[e]	9	1.5
control	7.4[j]	3.5[j]	9[k]	25[k]	5[j]	0.003[l]	1.4[m]	3[k]

[a] For details of antiviral assays, see ref 1. [b] ELISA. [c] Plaque reduction. [d] Yield reduction (EC_{90}, μM). [e] The HIV-1 assay in MT-4 cells showed[8] a similar pattern of antiviral effect: EC_{50} 26 μM (**1a** + **2a**), 13 μM (**1a**), and 50 μM (**2a**). [f] Optical purity 95−96%. [g] EC_{50} 0.13 μM in the presence of 10 μM coformycin. [h] The same value was obtained in the presence of 10 μM coformycin. [i] EC_{50} of (*S*)-hypoxanthine derivative **5** was >50 μM. [j] Ganciclovir. [k] Acyclovir. [l] AZT. [m] Zalcitabine (ddC).

Table 2. Hydrogen Bonds (Å)[a]

donor	H	acceptor	D−H	H···A	D···A	angle (deg)
N1A	H1A	Cl2a	0.81(6)	2.29(6)	3.057(3)	157(5)
N1B	H1B	Cl1	0.88(5)	2.15(5)	3.014(4)	165(4)
N10A	H10A	N7B	0.80(6)	2.28(6)	3.028(5)	157(5)
N10A	H10B	Cl1a	0.79(6)	2.39(6)	3.106(4)	152(5)
N10B	H10C	N7A	0.94(4)	2.03(5)	2.934(5)	162(4)
N10B	H10D	Cl2	0.91(5)	2.25(5)	3.131(4)	162(4)
O16A	H16A	O16Ab	1.11(9)	1.83(9)	2.793(5)	141(7)

[a] Translation symmetry codes: $a = 1/2+x, -1/2+y, z$; $b = 1/2-x, -1/2+y, -z$.

each exhibit a different enantioselectivity toward nucleoside analogues.[13] For example, enantioselectivity (or lack of it) of carbovir and zalcitabine (ddC) is determined by enzymes involved in the phosphorylation pathway.[14,15] Nevertheless, target viral polymerases (e.g., HSV-1 and HBV) can also respond differently toward enantiomeric triphosphates.[16,17]

(±)-Synadenol (**1a** + **2a**) is unique in this aspect of antiviral activity since its enantioselectivity is completely lost with HCMV, VZV, and, to a large extent, HBV, HSV-1, and HSV-2, but it is reversed in the case of HIV-1 and EBV (Table 1). To the best of our knowledge, such varied pattern of enantioselectivity has not been observed with any broad-spectrum antiviral agent. Currently, it is assumed that triphosphates of enantiomers **1a** and/or **2a** are the actual inhibitors of viral DNA polymerases. The loss of enantioselectivity toward HCMV, VZV, and HBV indicates a little difference in the activation capability of enantiomers **1a** and **2a** and inhibition of the target polymerases by the respective triphosphates. The differences between enantiomers are more accentuated in the case of HBV, HSV-1, HSV-2, and, particularly, HIV-1 and EBV (Table 1). These results suggest that stereorecognition patterns of enzymes responsible for activation (phosphorylation) or the respective viral DNA polymerases toward enantiomers **1a** and **2a** could vary significantly with the type of virus involved. The mechanism of action of methylenecyclopropane analogues **1a** and **2a** may then vary not only with the class of virus involved (HCMV, VZV, and HBV vs HIV) but also within the same class (HCMV, HSV-1, HSV-2, VZV, or EBV). These intriguing mechanistic possibilities remain to be fully explored.

Experimental Section

General Methods. See ref 1. The circular dichroism (CD) spectra were determined[5] using JASCO J-600 CD spectrometer in 0.05 M Na_2HPO_4 (pH 7.0).

(−)-Synadenol (1a) and (+)-Synhypoxanthol (5). A suspension of (±)-synadenol (**1a** + **2a**; 200 mg, 0.92 mmol) in 0.05 M Na_2HPO_4 (pH 7.5, 100 mL) was briefly sonicated. Adenosine deaminase from calf intestine (type II, Sigma Chemical Co., St. Louis, MO; 30 mg, 45 units) was added, and the mixture was stirred at room temperature. The progress of the reaction was followed by TLC (CH_2Cl_2−MeOH, 9:1). The deamination stopped after 72 h, and the ratio of (**1a** + **2a**):**5** was 7:3 as determined by UV spectrophotometry of the eluted spots. Addition of another two portions of enzyme (30 mg every 24 h) did not improve this ratio. The mixture was lyophilized, and the resultant product was sonicated with several portions of CH_2Cl_2−MeOH (1:1, first 100 mL and then 40 mL) until no UV absorption was detectable in the solvent. The filtrate and washings were combined and evaporated. The residue was adsorbed on silica gel (4 g) and loaded on a column made of the same material. Chromatography using CH_2Cl_2−MeOH (9:1) as an eluent afforded (−)-synadenol (**1a**) (135 mg, 66.5%), $[\alpha]^{25}_D$ −59.1° (*c* 0.04, MeOH), and elution with CH_2Cl_2−MeOH (4:1) gave (+)-synhypoxanthol (**5**) (85 mg), $[\alpha]^{25}_D$ 91.8° (*c* 0.1, MeOH).

The obtained (−)-synadenol (**1a**) (135 mg, 0.62 mmol) was subjected to a second round of deamination (60 mg, 90 units of enzyme) under the conditions described above. TLC showed a ratio of (**1a** + **2a**):**5** as 7:3 after 40 h. Prolonged reaction time did not improve the conversion. Workup followed by chromatography as described above gave optically enriched (−)-synadenol (**1a**), 95 mg, 47.5%, $[\alpha]^{25}_D$ −112.0° (*c* 0.095, MeOH), and (+)-synhypoxanthol (**5**), 40 mg, $[\alpha]^{25}_D$ 89.0° (*c* 0.1, MeOH). The combined portions of product **5** (125 mg) were rechromatographed on silica gel using CH_2Cl_2−MeOH (4:1) to afford optically pure (+)-synhypoxanthol (**5**), 96 mg, 47.8%, $[\alpha]^{25}_D$ 112.5° (*c* 0.08, MeOH), as a white solid: mp 235−240 °C; UV (EtOH) λ_{max} 228 nm (ϵ 31 300); IR (KBr) 3450 (OH), 1600 and 1670−1690 (purine ring and olefin), 1040 cm^{-1} (cyclopropane ring); ^{1}H NMR (DMSO-d_6) δ 1.21 (ddd, 1 H, ^2J = 8.4 Hz, $^3J_{trans}$ = 5.4 Hz, ^4J = 1.8 Hz) and 1.48 (td, 1 H, ^2J = $^3J_{cis}$ = 8.7 Hz, ^4J = 1.5 Hz, $H_{3'}$), 2.12 (dqd, 1 H, $^3J_{cis}$ = 9.0 Hz, $^3J_{trans}$ = ^3J = 5.4 Hz, ^4J = 1.8 Hz, $H_{4'}$), 3.28 (dt, overlapped with H_2O, 1 H, ^2J = 11.1 Hz, ^3J = 7.2 Hz) and 3.72 (dd, 1 H, ^2J = 10.8 Hz, ^3J = 5.1 Hz, $H_{5'}$), 5.09 (t, 1 H, ^3J = 4.8 Hz, OH), 7.33 (d, 1 H, ^4J = 1.8 Hz, $H_{1'}$), 8.07 (s, 1 H, H_2), 8.69 (s, 1 H, H_8), 12.41 (s, 1 H, NH); ^{13}C NMR 6.67 ($C_{3'}$), 19.62 ($C_{4'}$), 63.11 ($C_{5'}$), 110.50 ($C_{1'}$), 117.58 ($C_{2'}$), 124.18 (C_5), 137.56 (C_8), 146.61 (C_2), 147.19 (C_4), 156.97 (C_6); EI-MS 217 (M − H, 26.3), 200 (M − H_2O, 58.6), 187 (40.5), 159 (11.7), 148 (16.1), 135 (hypoxanthine − H, 100.0). Anal. ($C_{10}H_{10}N_4O_2$·0.6H_2O) C, H, N.

In another experiment (±)-synadenol (**1a** + **2a**) (450 mg, 2.07 mmol) furnished optically enriched (−)-enantiomer **1a** (200 mg, 44.4%) and (+)-synhypoxanthol (**5**) (200 mg, 44.4%). The combined portions of optically enriched adenine analogue **1a** (280 mg, 1.29 mmol) from both experiments were incubated with adenosine deaminase (100 mg, 150 units) in phosphate buffer (250 mL) as described above. The ratios of **1a**:**5** were 94:6 after 24 h and 93:7 after 48 h. Workup and purification as described above gave optically pure **1a** (250 mg, 38.5% overall yield based on starting material **1a** + **2a**, 650 mg, 2.99 mmol), $[\alpha]^{25}_D$ −120.0° (*c* 0.075, MeOH), and optically enriched hypoxanthine analogue **5** (20 mg, 7.1%), $[\alpha]^{25}_D$ 60.6° (*c* 0.11, MeOH). Optical purity of **1a** was 95% as determined by chiral HPLC (Figure 1, panel A): mp 237−238 °C; UV (EtOH) λ_{max} 277 nm (shoulder, ϵ 8 800), 260 (ϵ 12 300), 227 (ϵ 26 900); IR,

^{1}H NMR, and EI-MS were identical to the racemic material[1] **1a** + **2a**. Anal. ($C_{10}H_{11}N_5O$), C, H, N.

***O*-Acetyl-(+)-synhypoxanthol (6).** A mixture of (+)-synhypoxanthol (**5**) (131 mg, 0.6 mmol) and acetic anhydride (1.0 mL, 10.6 mmol) in pyridine (10 mL) was stirred at room temperature for 16 h. Volatile components were removed in vacuo, and the residue was passed through a short silica gel column using CH_2Cl_2−MeOH (92:8) as an eluent to give acetate **6** (155 mg, 99%): mp 234−237 °C; $[\alpha]^{25}_D$ +108.6° (*c* 0.084, MeOH); UV (EtOH) λ_{max} 227 nm (ϵ 27 300); IR (KBr) 3460 and 3140 (NH), 1735 (ester), 1695 and 1595 (hypoxanthine ring and olefin), 1040 cm^{-1} (cyclopropane); ^{1}H NMR (DMSO-d_6) δ 1.38 (ddd, 1 H, ^2J = 11.7 Hz, $^3J_{trans}$ = 5.4 Hz, ^4J = 1.8 Hz) and 1.63 (td, 1 H, ^2J = $^3J_{cis}$ = 8.9 Hz, ^4J = 1.5 Hz, H$_{3'}$), 1.88 (s, 3 H, CH$_3$), 2.32 (dqd, 1 H, $^3J_{cis}$ = 9.0 Hz, $^3J_{trans}$ = ^3J = 5.4 Hz, ^4J = 1.8 Hz, H$_{4'}$), 3.93 (dd, 1 H, ^2J = 11.3 Hz, ^3J = 7.4 Hz) and 4.14 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 6.9 Hz, H$_{5'}$), 7.37 (q, 1 H, ^4J = 1.8 Hz, H$_{1'}$), 8.06 (s, 1 H, H$_2$), 8.36 (s, 1 H, H$_8$), 12.40 (bs, 1 H, NH); ^{13}C NMR 7.79 (C$_{3'}$), 15.89 (CH$_3$), 20.89 (C$_{4'}$), 65.87 (C$_{5'}$), 111.56 (C$_{1'}$), 117.03 (C$_{2'}$), 124.19 (C$_5$), 137.65 (C$_8$), 146.68 (C$_2$), 147.42 (C$_4$), 156.99 (C$_6$), 170.53 (CO, acetate); EI-MS 260 (M, 26.1), 218 (M − Ac + H, 5.4), 201 (M − OAc, 66.5), 174 (15.6), 137 (hypoxanthine + H, 100.0); HRMS calcd for $C_{12}H_{12}N_4O_3$ M 260.0909, found M 260.0906. Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

(+)-(*Z*)-6-Chloro-9-((2-(acetoxymethyl)cyclopropylidene)methyl)purine (7). *N,N*-Dimethylaminochloromethyleneammonium chloride in $CHCl_3$ (0.2 M, 3.5 mL, 8.7 mmol) was added to a suspension of acetate **6** (150 mg, 0.58 mmol) in $CHCl_3$ (15 mL). The mixture was refluxed for 1 h. The solvent was evaporated, and the residue was chromatographed on a silica gel column using CH_2Cl_2−MeOH (96:4) to give product **7** (141 mg, 87.8%): mp 122−125 °C; $[\alpha]^{25}_D$ 93.9° (*c* 0.095, MeOH); UV (EtOH) λ_{max} 260 nm (shoulder, ϵ 8 200), 228 (ϵ 28 500); IR (KBr) 1730 (ester), 1590 and 1563 (purine ring and olefin), 1030 cm^{-1} (cyclopropane); ^{1}H NMR (DMSO-d_6) δ 1.40 (ddd, 1 H, ^2J = 9.2 Hz, $^3J_{trans}$ = 5.1 Hz, ^4J = 2.0 Hz) and 1.73 (td, 1 H, ^2J = $^3J_{cis}$ = 9.0 Hz, ^4J = 1.9 Hz, H$_{3'}$), 2.04 (s, 3 H, CH$_3$), 2.26−2.37 (m, 1 H, H$_{4'}$), 3.77 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 8.7 Hz) and 4.53 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 5.7 Hz, H$_{5'}$), 7.55 (q, 1 H, ^4J = 1.8 Hz, H$_{1'}$), 8.74 and 8.76 (2s, 1 H each, H$_2$ and H$_8$); ^{13}C NMR 7.46 (C$_{3'}$), 15.98 (CH$_3$), 20.80 (C$_{4'}$), 66.36 (C$_{5'}$), 111.11 (C$_{1'}$), 116.25 (C$_{2'}$), 131.41 (C$_5$), 142.32 (C$_8$), 150.32 (C$_4$), 151.18 (C$_2$), 152.44 (C$_6$), 170.56 (CO, acetate); EI-MS 280 and 278 (M, 7.3, 22.0), 238 and 236 (M − Ac + H, 2.6, 7.0), 221 and 219 (M − OAc, 36.2, 81.1), 207 (8.9), 183 (8.4), 157 and 155 (6-chloropurine + H, 29.4, 85.9), 82 (63.0), 43 (Ac, 100.0); HRMS calcd for $C_{12}H_{11}{}^{35}ClN_4O_2$ M 278.05705, found 278.0568. Anal. ($C_{12}H_{11}ClN_4O_2$) C, H, Cl, N.

(+)-Synadenol (2a). A mixture of compound **7** (130 mg, 0.47 mmol) in methanolic ammonia (20%, 70 mL) was heated in a stainless steel bomb at 100 °C for 18 h. After cooling, the contents were evaporated. The residue was chromatographed on silica gel using CH_2Cl_2−MeOH (9:1 → 85:15) to give (*S*)-(+)-synadenol (**2a**; 91 mg, 90%) after drying at <0.01 mmHg and 100 °C for 2 h. The optical purity of **2a** was 96% as determined by chiral HPLC (Figure 1, panel B): mp 233−235 °C (modification change between 213 and 230 °C); $[\alpha]^{25}_D$ 123.0° (*c* 0.073, MeOH); UV (EtOH) λ_{max} 277 nm (shoulder, ϵ 8 600), 261 (ϵ 12 200), 227 (ϵ 26 700); IR, ^{1}H NMR, and MS were identical to the racemic compound[1] **1a** + **2a**. Anal. ($C_{10}H_{11}N_5O$) C, H, N.

Ethyl (±)-Methylenecyclopropanecarboxylate (9). Ethanol (2.0 mL) was added to a mixture of ethyl 2-bromo-2-methylcyclopropanecarboxylate[10] (70.00 g, 0.34 mol), ether (250 mL), and sodium hydride (50% in mineral oil, 32.64 g, 0.68 mol) with stirring at room temperature. Within a few minutes, exothermic reaction started resulting in a vigorous refluxing of ether. Another portion of ethanol (2.0 mL) was added after 1 h, and the reaction mixture was refluxed for 16 h. After the mixture had cooled, pentane (150 mL) was added and the mixture was filtered through a Celite pad. The insoluble portion was washed with pentane (3 × 50 mL). The combined filtrate and washings were concentrated, and the crude product was distilled to give compound **9** (36.60 g, 85.8%). The ^{1}H NMR spectrum was identical to that reported.[10]

(±)-Methylenecyclopropanecarboxylic Acid (10). Potassium hydroxide (9.82 g, 175 mmol) was added to a mixture of ester **9** (12.61 g, 0.1 mol) in MeOH−H_2O (4:1, 120 mL) with stirring and ice cooling. The stirring was continued at room temperature for 18 h. Volatile components were removed in vacuo, and the residue was dissolved in water (60 mL). The pH was adjusted to 2.0 by a cautious addition of 4 M HCl with stirring and ice cooling. The resultant solution was extracted with CH_2Cl_2 (10 × 100 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give carboxylic acid **10** (9.64 g, 98.3%) as a colorless oil: ^{1}H NMR (CDCl$_3$) δ 1.72 (ddt, 1 H, ^2J = 9.0 Hz, $^3J_{cis}$ = 8.3 Hz, ^4J = 2.4 Hz) and 1.89 (ddt, 1 H, ^2J = 9.3 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 2.4 Hz, CH$_2$), 2.27 (ddt, 1 H, $^3J_{cis}$ = 8.3 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 2.1 Hz, CH), 5.55−5.59 (m, 2 H, ^4J = 2.1 Hz, =CH$_2$), 9.5 (bs, 1 H, CO$_2$H).

***N*-[(*R*)-1′-Phenyl-2′-hydroxyethyl]-(1*S*)-methylenecyclopropanecarboxamide (11) and *N*-[(*R*)-1′-Phenyl-2′-hydroxyethyl]-(1*R*)-methylenecyclopropanecarboxamide (12).** The described procedure[9] was followed with carboxylic acid **10** (9.63 g, 98.2 mmol), triethylamine (30.1 mL, 0.216 mol), isobutyl chloroformate (12.7 mL, 98.2 mmol), and (*R*)-phenylglycinol (13.47 g, 98.2 mmol) in THF (400 mL), but the separation of diastereoisomers **11** and **12** was modified. Several chromatographic runs on silica gel columns using CH_2Cl_2−THF (95:5 → 85:15) followed by CH_2Cl_2−MeOH (95:5 → 9:1) afforded the *R,R*-diastereoisomer **12** (8.78 g, 41.2%) and the *R,S*-diastereoisomer **11** (8.34 g, 39.4%) as white solids. The reported[9] combined yield of both diastereoisomers was 58%.

***S,R*-Isomer 11:** mp 133−136 °C; $[\alpha]^{23}_D$ −149.4° (*c* 0.87, EtOH); ^{1}H NMR (CDCl$_3$) δ 1.65 (tt, 1 H, ^2J = $^3J_{cis}$ = 9.0 Hz, ^4J = 2.4 Hz) and 1.75 (ddt, 1 H, ^2J = 9.0 Hz, $^3J_{trans}$ = 5.0 Hz, ^4J = 2.4 Hz, CH$_2$ of cyclopropane), 2.21 (ddt, 1 H, $^3J_{cis}$ = 8.7 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 2.4 Hz, CH of cyclopropane), 2.52 (bs, 1 H, OH), 3.87 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 5.2 Hz) and 3.92 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 5.2 Hz, CH$_2$O), 5.07 (dt, 1 H, ^3J = 5.7 Hz, ^3J = 5.2 Hz, C**H**C$_6$H$_5$), 5.55−5.59 (m, 2 H, J = 2.1 Hz, =CH$_2$), 6.26 (bs, 1 H, NH), 7.26−7.42 (m, 5 H, C$_6$H$_5$).

***R,R*-Isomer 12:** mp 137−139 °C; $[\alpha]^{25}_D$ −139.0° (*c* 0.81, EtOH); ^{1}H NMR (CDCl$_3$) δ 1.63 (tt, 1 H, ^2J = $^3J_{cis}$ = 9.0 Hz, ^4J = 2.4 Hz) and 1.77 (ddt, 1 H, ^2J = 9.0 Hz, $^3J_{trans}$ = 4.7 Hz, ^4J = 2.4 Hz, CH$_2$ of cyclopropane), 2.19 (ddt, 1 H, $^3J_{cis}$ = 10.8 Hz, $^3J_{trans}$ = 4.8 Hz, ^4J = 2.4 Hz, CH of cyclopropane), 2.32−2.49 (bs, 1 H, OH), 3.85 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 4.2 Hz) and 3.91 (dd, 1 H, ^2J = 11.4 Hz, ^3J = 5.4 Hz, CH$_2$O), 5.07 (dt, 1 H, ^3J = 6.6 Hz, ^3J = 5.1 Hz, C**H**C$_6$H$_5$), 5.57−5.62 (m, 2 H, =CH$_2$), 6.27 (bs, 1 H, NH), 7.28−7.42 (m, 5 H, C$_6$H$_5$).

(1*R*)-(−)-Methylenecyclopropanecarboxylic Acid (8). The described procedure[9] was modified as follows. The *R,R*-diastereoisomer **12** (8.68 g, 39.95 mmol) in THF (150 mL) and aqueous H_2SO_4 (1 M, 150 mL) was refluxed for 48 h. After cooling, the mixture was evaporated to about half of the original volume. Saturated aqueous NaCl (80 mL) was added to the residue and extracted with ether (300 mL, then 4 × 80 mL). The extraction was monitored by TLC in hexane−ethyl acetate (1:1.5). The combined extracts were dried (Na_2SO_4) and evaporated leaving a yellow oil of compound **8** (3.92 g, 100%), $[\alpha]^{25}_D$ −17.0° (*c* 1.0, EtOH).

Ethyl (1*R*)-(−)-Methylenecyclopropanecarboxylate (13). The (*R*)-carboxylic acid **8** (3.92 g, 39.95 mmol) was stirred at room temperature in ethanolic HCl (1 M, 60 mL) for 24 h. Water (60 mL) was added, and the mixture was extracted with pentane (8 × 80 mL). The combined extracts were washed with water, saturated aqueous $NaHCO_3$, water, and brine (80 mL each). The extraction was monitored by TLC in hexane−ethyl acetate (19:1). After drying (Na_2SO_4) the solvents were distilled off at an atmospheric pressure, and the crude ester **13** was used directly in the next step.

Ethyl (2*R*)-2-Bromo-2-(bromomethyl)-(1*S*)-cyclopropanecarboxylate and Ethyl (2*S*)-2-Bromo-2-(bromomethyl)-(1*S*)-cyclopropanecarboxylate (14). Bromine (2.25

mL, 43.67 mmol) was added to a solution of (*R*)-ester **13** (39.95 mmol) from the preceding experiment in CCl_4 (80 mL) over 15 min at 0 °C with stirring. After 0.5 h, TLC (hexane−ethyl acetate, 9:1) showed a complete reaction. Volatile components were removed in vacuo to afford a yellow oil of compound **14** (11.02 g) which was used in the next step. The ^{1}H NMR spectrum was identical to that of the racemic mixture[1] except for the *E*/*Z*-ratio which was 1:9. Chiral HPLC on Chiralpak AD in hexane−2-propanol (9:1, 1 mL/min, detection at 220 nm) showed no detectable amount of the corresponding 1*R*,2*S*- and 1*R*,2*R*-diastereoisomers. The retention times (min) of all four diastereoisomers[1] were as follows: 4.34 (1*S*,2*S*), 4.47 (1*R*,2*S*), 5.65 (1*S*,2*R*), and 6.02 (1*R*,2*R*).

(2*R*)-2-Bromo-2-(bromomethyl)-(1*S*)-(hydroxymethyl)-cyclopropane and (2*S*)-2-Bromo-2-(bromomethyl)-(1*S*)-(hydroxymethyl)cyclopropane (15). The procedure described for the preparation of a mixture of all four diastereoisomers of ethyl 2-bromo-2-(bromomethyl)cyclopropanecarboxylate[3] was followed with 1*S*,2*R*- and 1*S*,2*S*-diastereoisomers **14**. Chromatography on a silica gel column using hexane−ethyl acetate (4:1 → 1.5:1) as eluents gave compound **15** (7.31 g, 75% overall yield based on amide **12**). The ^{1}H NMR spectrum was identical to that reported previously[3] except for the diastereoisomeric composition.

(2*R*)-2-Bromo-2-(bromomethyl)-(1*S*)-(acetoxymethyl)-cyclopropane and (2*S*)-2-Bromo-2-(bromomethyl)-(1*S*)-(acetoxymethyl)cyclopropane (16). Again, the described procedure[3] was followed. Acetylation of **15** (7.31 g, 29.97 mmol) gave acetate **16** (8.23 g, 96% yield) which was used as such in the next step. The ^{1}H NMR spectrum was identical to that reported[3] except for the diastereoisomeric composition.

(*R*),(*Z*)-9-((2-(Acetoxymethyl)cyclopropylidene)methyl)-adenine (17) and (*R*),(*E*)-9-((2-(Acetoxymethyl)cyclopropylidene)methyl)adenine (18). The alkylation−elimination procedure[3] performed with adenine (43 mg, 0.32 mmol) and 2-bromo-2-(bromomethyl)-(1*S*)-(acetoxymethyl)cyclopropanes (**16**; 45 mg, 0.16 mmol) in DMF (5 mL) in the presence of K_2CO_3 (221 mg, 1.60 mmol) at 100 °C for 24 h afforded the title compounds **17** and **18** (35 mg, 85.8% yield) after column chromatography in CH_2Cl_2−MeOH (95:5); mp 160−174 °C; UV (EtOH) λ_{max} 277 nm (shoulder, ϵ 7 600), 261 (ϵ 11 200), 226 (ϵ 19 300), 197 (ϵ 7 600). This product was used directly in the next step.

(*R*)-(−)-Synadenol (1a) and *R*,*E*-Isomer 19. The mixture of **17** and **18** (30 mg, 0.11 mmol) was stirred in methanolic ammonia (20%, 10 mL) at room temperature for 16 h. Volatile components were evaporated, and the residue was dried at <0.01 mmHg at 78 °C for 1 h leaving a mixture of (*R*)-(−)-synadenol (**1a**) and the *E*-isomer **19** as a white solid (25 mg, 100%). The ^{1}H NMR spectra were identical to those of the respective racemic compounds.[1] The chiral HPLC (Figure 1, panel D) showed the ratio of **1a**:**19** as 1.5:1. The ee values for **1a** and **19** were 98.4% and 98.5%, respectively.

Crystal Data and Data Collection. For an X-ray diffraction study (−)-synadenol (**1a**; 95% ee) was further enriched by exhaustive deamination. (−)-Synadenol (**1a**) obtained as described above (65 mg, 0.3 mmol) was incubated with adenosine deaminase in phosphate buffer (60 mL). The recovered **1a** had 99.2% ee as determined by chiral HPLC. Crystals of (−)-synadenol hydrochloride for X-ray diffraction were obtained by slow crystallization of an ethanol solution of approximately equal molar amounts of (−)-synadenol and hydrochloric acid. The specimen chosen for preliminary study and data collection was a colorless thin needle of dimensions 1.00 × 0.075 × 0.080 mm. Unit cell parameters were determined from refinement of all reflections: $a = 20.986(1)$, $b = 4.951(0)$, $c = 22.845(1)$ Å; $\beta = 99.30(0)°$; Mo Kα radiation $\lambda = 0.71073$ Å; $F(000) = 1056$; room temperature; space group *C*2; $V = 2342$ Å^3; $Z = 8$; D(calcd) = 1.439 g/cm^3. Nonius Kappa CCD diffractometer and Kappa CCD server software were employed. Processing and data reduction were carried out using the program Denzo-SMN.[18] No absorption corrections were necessary. A total of 4015 reflections were measured, including Friedel pairs; $2\theta_{max} = 52°$; $h = -25{:}25$, $k = -5{:}6$, $l = -27{:}28$.

Structure Solution, Absolute Configuration, and Refinement. The structure was solved by direct methods using the program[19] SHELXS-97 and refined[20] using SHELXL-97. A total of 3132 reflections with $I > 2\sigma(I)$ were included in the refinement. All hydrogen atoms (except one at O16b and one at C15b of the B molecule) were found from the difference map. All the atoms were refined (non-hydrogen atoms anisotropically and hydrogen isotropically; 416 parameters). Final $R = 0.0439$. The highest peak in the final difference map was 0.17 e/Å^3, lowest −0.18 e/Å^3. The absolute configuration was established by refinement of the Flack parameter:[21] $x = -0.03(8)$. The discrepancy factor for the inverted structure was $R = 0.0451$, and the Flack parameter was 0.97(8).

Antiviral Assays. Assays used for biological evaluation of (±)-synadenol (**1a** + **2a**), (*R*)-(−)-synadenol (**1a**), and (*S*)-(+)-synadenol (**2a**) were described in detail previously.[1] The following virus-infected cultures were employed: human cytomegalovirus (HCMV) in human foreskin fibroblasts (HFF; plaque and yield reduction assay), herpes simplex virus 1 (HSV-1) in BSC-1 cells (ELISA) and in Vero cells (plaque reduction), herpes simplex virus 2 (HSV-2) in Vero cells (plaque reduction), Epstein-Barr virus (EBV) in H-1 cells, human immunodeficiency virus 1 (HIV-1) in CEM-SS cells, and hepatitis B virus (HBV) in 2.2.15 cell culture. The varicella zoster virus (VZV) was assayed by growth inhibition of human embryonic lung (HEL) cells. The results are summarized in Table 1.

VZV Growth Inhibition Assay. Confluent HEL cells in 12-well plates were inoculated with 30−40 pfu/well of virus. After 1-h adsorption at 37 °C, the inoculum was replaced with medium containing 10% FBS and tested compounds at various concentrations. Each dosage was duplicated. The infected cells were harvested at 72-h postinfection. The cells were lysed by “freeze−thaw”, and the lysate was treated with protease K and RNase. The DNA was then subjected to slot−blot assay as described previously.[22] The viral DNA was detected by hybridization with ^{32}P-labeled DNA fragment of VZV thymidine kinase (TK) gene. Autoradiographic results were quantitated by personal densitometer SI (Molecular Dynamics, Sunnyvale, CA). The same membrane was stripped and rehybridized with human b-actin gene fragment. The viral DNA amount was subsequently normalized by VZV DNA/human actin DNA. The concentration of the tested compounds, which inhibited 50% of viral DNA synthesis compared to untreated control, was defined as EC_{50}.

Acknowledgment. We thank the Central Instrumentation Facility, Department of Chemistry, Wayne State University (Dr. Robin J. Hood, Director), for mass spectra. The work described herein was supported by U.S. Public Health Service Research Grants RO1-CA32779 (J.Z.) from the National Cancer Institute and RO1-CA44358 (Y.-C.C.), U19-Al31718, and RO1-Al33332 (J.C.D.), and RO1-Al40392 (A.C.) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, and by the National Cancer Institute of Canada with funds from the Canadian Cancer Society (N.C.).

Supporting Information Available: Crystallographic data of (*R*)-(−)-synadenol (**1a**) (hydrochloride and free base) and Figure 1 (14 pages); tables of observed and calculated structure factors (20 pages). Ordering information is given on any current masthead page.

References

(1) Qiu, Y.-L.; Ksebati, M. B.; Ptak, R. G.; Fan, B. Y.; Breitenbach, J. M.; Drach, J. C.; Lin, J.-S.; Cheng, Y.-C.; Kern, E. R.; Zemlicka, J. (*Z*)- and (*E*)-2-(Hydroxymethylcyclopropylidene)methyladenine and -guanine - New Nucleoside Analogues with a Broad-Spectrum Antiviral Activity. *J. Med. Chem.* **1998**, *41*, 10−23.

(2) Qiu, Y.-L.; Ptak, R. G.; Fan, B. Y.; Breitenbach, J. M.; Drach, J. C.; Lin, J.-S.; Cheng, Y.-C.; Kern, E. R.; Zemlicka, J. (*Z*)- and (*E*)-2-(Hydroxymethylcyclopropylidene)methylpurines and pyrimidines as Antiviral Agents. *Antiviral Chem. Chemother.* **1998**, *9*, 341−352.
(3) Qiu, Y.-L.; Zemlicka, J. A New Efficient Synthesis of Antiviral Methylenecyclopropane Analogues of Purine Nucleosides. *Synthesis* **1998**, 1447−1452.
(4) Qiu, Y.-L.; Zemlicka, J. 3,5′-Anhydrosynadenol - a New type of Anhydronucleoside Analogue and Polycyclic System Comprising a Methylenecyclopropane Moiety. *Angew. Chem., Int. Ed.* **1998**, *37*, 1440−1441.
(5) Megati, S.; Goren, Z.; Silverton, J. V.; Orlina, J.; Nishimura, H.; Shirasaki, T.; Mitsuya, H.; Zemlicka, J. (*R*)-(−)- and (*S*)-(+)-Adenallene: Synthesis, Absolute Configuration, Enantioselectivity of Antiretrovial Effect, and Enzymic Deamination. *J. Med. Chem.* **1992**, *35*, 4098−4104.
(6) 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.
(7) Runge, W. Chiroptical Properties of Cyclopropane Derivatives. In *The Chemistry of the Cyclopropyl Group*; Rappoport, Z., Ed.; John Wiley & Sons: New York, 1987; pp 27−99.
(8) Cheng, C.; Shimo, T.; Somekawa, K.; Baba, M. 9-Hydroxycyclopropylidene-methylenyladenine: The Design, Facile Synthesis, Isomer Separation and Anti-HIV Activities. *Tetrahedron* **1998**, *54*, 2031−2040.
(9) Lai, M.-t.; Liu, L.-d.; Liu, H.-w. Mechanistic Study of the Inactivation of General Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin A. *J. Am. Chem. Soc.* **1991**, *113*, 7388−7397.
(10) Gajewski, J. J.; Burka, L. T. Axially Dissymmetric Molecules. Characterization of the Four 1-Carbethoxy-4-methylspiropentanes. *J. Org. Chem.* **1970**, *35*, 2190−2196.
(11) Cahn, R. S.; Ingold, C. K.; Prelog, V. Specification of Molecular Chirality. *Angew. Chem., Int. Ed.* **1966**, *5*, 385−415.
(12) Taylor, R.; Kennard, O. The Molecular Structures of Nucleosides and Nucleotides. 1. The Influence of Protonation on the Geometries of Nucleic Acid Constituents. *J. Mol. Struct.* **1982**, *78*, 1−28.
(13) Furman, P. A.; Wilson, J. E.; Reardon, J. E.; Painter, G. R. The Effect of Absolute Configuration on the Anti-HIV and Anti-HBV Activity of Nucleoside Analogues. *Antiviral Chem. Chemother.* **1995**, *6*, 345−355.
(14) Miller, W. H.; Daluge, S. M.; Garvey, E. P.; Hopkins, S.; Reardon, J. E.; Boyd, F. L.; Miller, R. L. Phosphorylation of Carbovir Enantiomers by Cellular Enzymes Determines the Stereoselectivity of Antiviral Activity. *J. Biol. Chem.* **1992**, *267*, 21220−21224.
(15) Shafiee, M.; Boudou, V.; Griffon, J.-F.; Pompon, A.; Gosselin, G.; Eriksson, S.; Imbach, J.-L.; Maury, G. Study of the Enantioselectivity of Enzymes Involved in Nucleoside Analogue Metabolism: Deoxycytidine Kinase. *Nucleosides Nucleotides* **1997**, *16*, 1767−1770 and references cited therein.
(16) Vere Hodge, R. A. The Mode of Action of Famciclovir Including Its Conversion to Penciclovir. *Antiviral Chem. Chemother.* **1993**, *4*, 67−84.
(17) Shaw, T.; Mok, S.; Locarnini, S. Inhibition of Hepatitis B Virus DNA Polymerase and Metabolic Basis for Selective Inhibition of HBV Replication by Penciclovir. *Hepatology* **1996**, *24*, 996−1002.
(18) Otwinowski, Z.; Minor, W. Processing of X-Ray Diffraction Data in Oscillation Mode. *Methods Enzymol.* **1997**, *276*, 307−326.
(19) Sheldrick, G. M. *SHELXS-97. Program for the Solution of Crystal Structures*; Univ. of Gottingen: Germany, 1997.
(20) Sheldrick, G. M. *SHELXL-97. Program for the Refinement of Crystal Structures*; Univ. of Gottingen: Germany, 1997.
(21) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876−881.
(22) Yao, G.; Liu, S. H.; Chou, E.; Kukhanova, M.; Chu, C. K.; Cheng, Y.-C. Inhibition of Epstein-Barr Virus Replication by a Novel *L*-Nucleoside, 2-Fluoro-5-methyl-β-*L*-arabinofuranosyluracil. *Biochem. Pharmacol.* **1996**, *51*, 941−947.

JM980323U