

(Z)- and (E)-2-(1,2-Dihydroxyethyl)methylenecyclopropane Analogues of 2'-Deoxyadenosine and 2'-Deoxyguanosine. Synthesis of All Stereoisomers, Absolute Configuration, and Antiviral Activity

Shaoman Zhou,[†] John C. Drach,[‡] Mark N. Prichard,[§] and Jiri Zemlicka^{*,†}

Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201-1379, Department of Biologic and Materials Science, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48018-3218, and Department of Pediatrics, The University of Alabama School of Medicine, Birmingham, Alabama 35233

Received February 2, 2009

Chiral *Z*- and *E*-stereoisomers of (1,2-dihydroxyethyl)methylenecyclopropane analogues of 2'-deoxyadenosine and 2'-deoxyguanosine were synthesized, and their antiviral activity was investigated. (*S*)-Methylenecyclopropylcarbinol (**16**) was converted in seven steps to reagents **26** and **27**, which were used for alkylation–elimination of adenine and 2-amino-6-chloropurine to get ultimately analogues **12a**, **12b**, **13a**, **13b**, **14a**, **14b**, **15a**, and **15b**. The enantiomeric series *ent*-**12a**, *ent*-**12b**, *ent*-**13a**, *ent*-**13b**, *ent*-**14a**, *ent*-**14b**, *ent*-**15a**, and *ent*-**15b** was obtained by similar procedures starting from (*R*)-methylenecyclopropylcarbinol (*ent*-**16**). The *Z*-isomer *ent*-**12b** was an inhibitor of two strains of human cytomegalovirus (HCMV) with EC₅₀ of 6.8 and 7.5 μM and of murine cytomegalovirus (MCMV) with EC₅₀ of 11.3 μM. It was less active against HCMV with mutated gene UL97. It inhibited Epstein–Barr virus (EBV) with EC₅₀ of 8 μM. The *E*-isomers *ent*-**15a**, *ent*-**13a**, and **15b** were less effective. All adenine analogues with the exception of the *Z*-isomers *ent*-**12a** and *ent*-**14a** were moderate substrates for adenosine deaminase.

Introduction

Methylenecyclopropane analogues of nucleosides are established antiviral agents¹ effective against β -herpesviruses (cytomegalovirus, CMV;^a human herpes virus 6, HHV-6) and γ -herpesviruses (Epstein–Barr virus, EBV; human herpes virus 8, HHV-8). In the first generation analogues, which have only a single hydroxymethyl group, the *Z*-isomers **1** (Chart 1) are most effective whereas the *E*-isomers **2** are active only exceptionally. In the second generation group, the antiviral activity is more narrow but the trend was similar. The *Z*-isomers **3** were effective against CMV and EBV, whereas the anti-EBV effect was also found in some of the *E*-isomers **4**. In this group of methylenecyclopropanes containing two geminal hydroxymethyl functions, the most potent analogue, cyclopropavir (**3b**), is now in preclinical development as a potential drug against human cytomegalovirus (HCMV) infections.^{2–5} Vicinal *cis*-bis-hydroxymethyl analogues **5a** and **5b** were ineffective as antiviral agents.⁶ Adenine *trans* analogue **6** was also synthesized, but antiviral activity has not been reported.⁷ Addition of a third hydroxymethyl group (*Z*- and *E*-isomers **7** and **8**) led to a loss of antiviral activity.⁸ Analogues **1** and **2** can be regarded as analogues of antiherpetic drug acyclovir (**9**), whereas bis-hydroxymethyl methylenecyclopropanes **3** and **4**, and particularly cyclopropavir (**3b**), are related to ganciclovir (**10**), a drug used against HCMV.

The analogy between C–O–C grouping of acyclovir (**9**) or ganciclovir (**10**) and methylenecyclopropane moiety was crucial in designing analogues **1–4**. It was therefore of interest whether this type of relationship can be extended to other antiviral nucleoside analogues. We determined that 9-[(2,3-dihydroxy-1-propoxy)methyl]guanine (**11**) would be a good starting point for such a design. Structurally, it is a positional isomer of ganciclovir (**10**) with a strong potency against herpes simplex viruses 1 and 2 (HSV-1, HSV-2) and HCMV.^{9,10} The *S*-enantiomer of **11** is more potent than the *R*-enantiomer. A design of methylenecyclopropane analogues based on compound **11** is stereochemically more complex. Thus, from a single heterocyclic base, eight stereoisomers can be derived given the two chiral centers present and taking *Z* and *E* isomerism into account (Chart 2, **12–15** and *ent*-**12** to *ent*-**15**). All 16 analogues comprising two bases, adenine and guanine, were synthesized and tested for antiviral activity. It is important to note that vertical relationships in Chart 2 (columns) are enantiomeric, whereas horizontal (rows) correspond to diastereoisomers or *Z,E*-isomers.

Synthesis

Enantiomeric (methylenecyclopropyl)methanols^{11–13} **16** and *ent*-**16** served as convenient starting materials for synthesis of all stereoisomeric analogues reported herein. Importantly, the originally assigned^{11,12} absolute configurations of **16** and *ent*-**16** were later reversed.¹³ In the 4'*S* series of analogues **12–15**, (*S*)-(+)-(methylenecyclopropyl)methanol (**16**) was first oxidized to the respective aldehyde **17** using oxalyl chloride and DMSO reagent¹² (Scheme 1). The crude aldehyde **17** was transformed to the diastereoisomeric cyanohydrin **18** using NaCN under phase transfer conditions¹⁴ in 54% yield. Hydrolysis gave the corresponding acid **19**, which in turn were converted to benzyl esters **20** and **21** by the action of benzyl bromide, NBu₄I, and K₂CO₃ in DMF. Both diastereoisomers were readily separated by column chromatography on silica gel to give the 1*S*,2*S*-

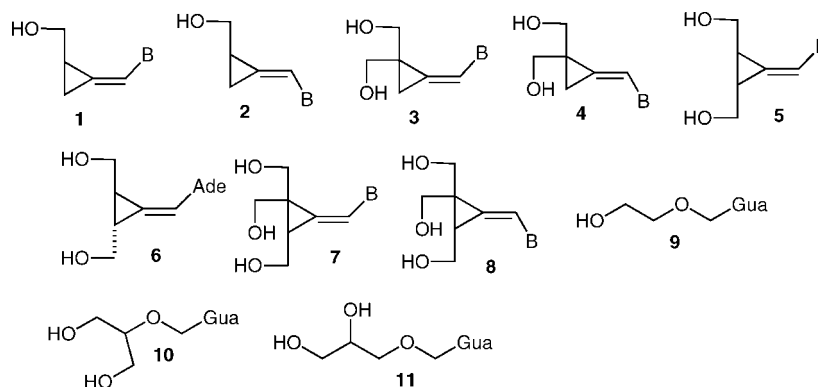
* To whom correspondence should be addressed. Telephone: (313) 578-4288. Fax: (313) 832-7294. E-mail: zemlicka@karmanos.org.

[†] Wayne State University School of Medicine.

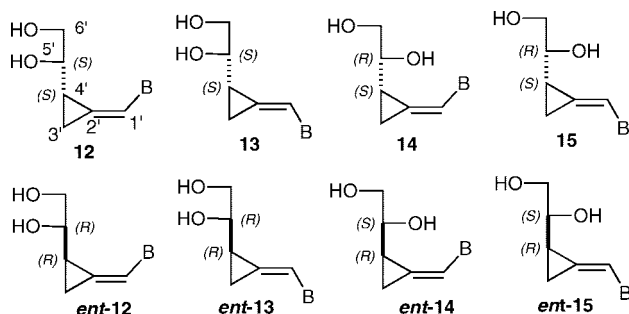
[‡] University of Michigan.

[§] The University of Alabama School of Medicine.

^a Abbreviations: CMV, cytomegalovirus; HCMV, human cytomegalovirus; MCMV, murine cytomegalovirus; UL97, gene for human cytomegalovirus phosphotransferase, pUL97; phosphotransferase enzyme; EBV, Epstein–Barr virus; EBV; HSV-1, herpes simplex virus 1; HSV 2, herpes simplex virus 2; HHV 6, human herpes virus 6; HHV-8, human herpes virus 8; VZV, varicella zoster virus; HFF, human foreskin fibroblasts; MEF, mouse embryonic fibroblasts; CPE, cytopathic effect; ADA, adenosine deaminase; NOE, nuclear Overhauser effect.

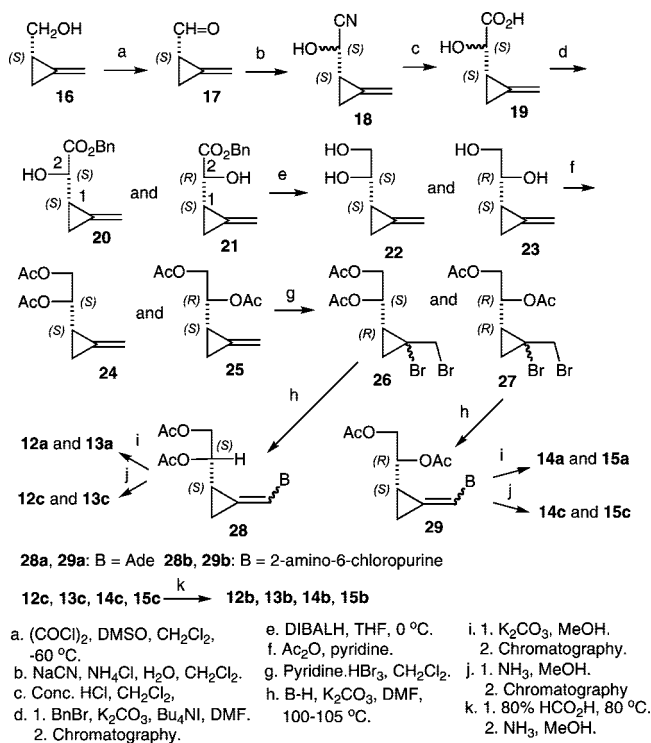
Chart 1^a

^a Series a: B = Ade. Series b: B = Gua. Series c: B = 2-amino-6-chloropurine.

Chart 2^a

^a Series a: B = Ade. Series b: B = Gua. Series c: B = 2-amino-6-chloropurine.

Scheme 1



stereoisomer **20** (35%) and 1*S*,2*R*-stereoisomer **21** (38%). Compounds **20** and **21** were reduced with diisobutylaluminum hydride (DIBALH) in hexanes to give diols **22** and **23** in 74% and 79% yields, respectively. Acetylation afforded diacetates **24** and **25** (84% and 86%). Bromination of **24** using pyridinium

Table 1. Chiral HPLC of Adenine Stereoisomers^a **12a**–**15a** and *ent*-**12a** to *ent*-**15a**

stereoisomer	<i>t</i> _R (min) ^b	ee (%) ^c
12a	10.62	95.3
13a	11.94	96.5
14a	11.69	99.0
15a	7.80	96.4
<i>ent</i> - 12a	8.10	100.0
<i>ent</i> - 13a	8.0	100.0
<i>ent</i> - 14a	7.21	100.0
<i>ent</i> - 15a	6.37	100.0

^a Chiralpak AD column, 25 cm × 0.46 cm, methanol, 1.0 mL/min, detection at 277 nm. ^b *t*_R, retention time. ^c ee, enantiomeric enhancement.

tribromide in CH₂Cl₂ furnished a mixture of the *Z/E*-isomers **26** (86%), whereas **25** gave dibromides **27** in 76% yield.

The 4'*S*,5'*S* series of analogues **12** and **13** were obtained using dibromide **26**. Alkylation–elimination protocol with adenine and **26** (K₂CO₃ in DMF at 100–105 °C) gave an inseparable mixture of the *Z*- and *E*-isomeric acetates **28a** in 56% yield. In a similar fashion, 2-amino-6-chloropurine and **26** afforded intermediates **28b** (57% yield). Deacetylation of **28a** with K₂CO₃ in methanol furnished, after chromatographic separation, the *Z*-isomer **12a** (36%) and *E*-isomer **13a** (49%). Deprotection of **28b** was performed using NH₃ in methanol to give the *Z*- and *E*-isomers **12c** and **13c** in 41% and 48% yields, respectively. Hydrolysis of **12c** and **13c** in 80% formic acid at 80 °C followed by treatment with NH₃ in methanol gave guanine *Z*- and *E*-isomers **12b** and **13b** (83% and 84%).

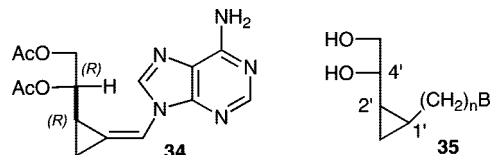
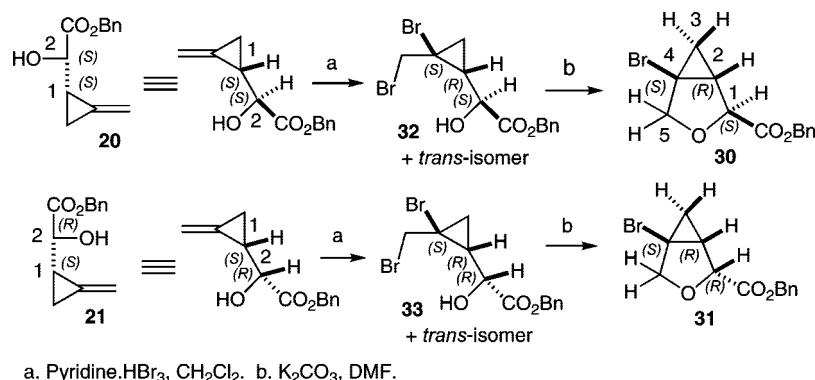
In the 4'*S*,5'*R* series **14** and **15**, reaction of adenine with dibromide **27** was performed under the conditions similar to those used for dibromide **26** to give the *Z/E* isomers **29a** (58%). 2-Amino-6-chloropurine and **27** gave the isomeric mixture **29b** (55% yield). Deacetylation of **29a** gave the *Z*- and *E*-isomers **14a** and **15a** in 35% and 48% yields, respectively, whereas **29b** afforded the *Z*- and *E*-isomers **14c** and **15c** (34% and 45%). Hydrolytic dechlorination furnished guanine analogues **14b** and **15b** (86% and 83%).

Synthesis of the enantiomeric series *ent*-**12** through *ent*-**15** started from (*R*)-(-)-(methylenecyclopropyl)methanol (*ent*-**16**) and followed the procedures outlined in Scheme 1 (*ent*-**16** through *ent*-**29**). The enantiomeric enhancement (ee) of adenine stereoisomers **12a**–**15a** and *ent*-**12a** through *ent*-**15a** determined by chiral HPLC was >95% (Table 1).

Assignment of Absolute Configuration and *Z/E* Isomerism

The absolute configuration of diastereoisomers **12**–**15** and *ent*-**12** to *ent*-**15** were assigned as follows. The configurations

Scheme 2



at the cyclopropane carbon atom C-4' of analogues were guaranteed by enantiomeric starting materials **16** and *ent*-**16** (see carbon C-1 in structures **20** and **21**). To establish configuration at the neighboring exocyclic carbon C-5' (C-2 of **20** and **21**), both esters were transformed to conformationally locked oxabicyclohexanes **30** and **31** (Scheme 2). Stereoisomer **20** was first converted to *cis*,*trans*-dibromide **32**, which was in turn cyclized to oxabicyclohexane **30** (35%). Similarly, compound **21** afforded isomeric derivative **31** via dibromide **33** in 32% yield. In both cases, only the *cis* isomers of **32** and **33** can undergo cyclization. A similar reaction of unprotected racemic methylenecyclopropyl alcohols with iodine followed by cyclization was described.¹⁵ Structure and configuration of both stereoisomers **30** and **31** followed from NMR spectroscopy including nuclear Overhauser effect (NOE) experiments. A strong NOE enhancement (2.79% and 3.07%) was found in compound **31** between the *cis*-configured H-1 and H-2, whereas none was present in isomer **30** where these hydrogens are *trans*. This established that absolute configuration is 1*S*,2*R* in **30** and 1*R*,2*R* in **31**, respectively. Consequently, the configurations of esters **20** and **21** are 1*S*,2*S* and 1*S*,2*R*. The enantiomers *ent*-**20** and *ent*-**21** have then opposite configurations. Thus, the absolute configurations of all four key intermediates **20**, **21**, *ent*-**20**, and *ent*-**21** were secured.

Dibromides **26**, **27**, *ent*-**26**, and *ent*-**27** obtained from these intermediates (Scheme 1) were used for synthesis of all stereoisomeric analogues. Consequently, two pairs of adenine *Z*- and *E*-enantiomers **12a**, *ent*-**12a** and **13a**, *ent*-**13a** were readily identified and their relationship with **14a**, **15a** and *ent*-**14a**, *ent*-**15a** must be diastereoisomeric. Similar conclusions apply for the respective guanine analogues.

As observed with other methylenecyclopropane analogues,¹⁶ the *cis*-(*Z*)-isomers were less polar, moving more quickly when chromatographed on silica gel than *trans*-(*E*)-isomers. Final isomeric assignment was confirmed by NMR spectroscopy (Table 2). The chemical shifts of H_{1'} and C_{3'} followed the trend observed earlier:^{2,16} H_{1'}(*E*) > H_{1'}(*Z*) and C_{3'}(*E*) > C_{3'}(*Z*). Nevertheless, the "usual" pattern of chemical shifts of H₈ and 5'-OH (H₈(*Z*) > H₈(*E*) and 5'-OH(*Z*) > 5'-OH(*E*)) held only for the 4'*S*,5'*S* series **12a**, **13a**, **12b**, and **13b**. In the 4'*S*,5'*R* series **14a**, **15a**, **14b**, and **15b**, no significant differences in these chemical shifts were found for the *Z*- or *E*-isomers. This indicates a lack of deshielding of H₈ of the *Z*-isomers **14a** and

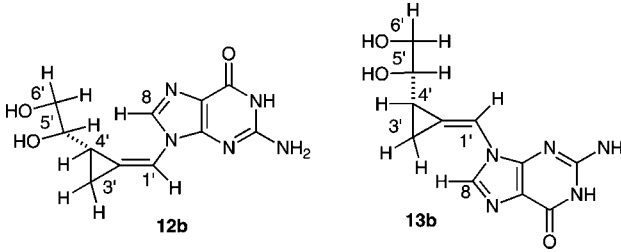
Table 2. Selected Chemical Shifts (δ , ppm) of Adenine and Guanine Analogues **12a–15a** and **12b–15b**

compd (isomer)	H _{1'}	H ₈	5'-OH	C _{3'}	C _{4'}
12a (<i>Z</i>)	7.38	8.96	5.23	6.7	20.8
13a (<i>E</i>)	7.49	8.46	4.83	8.7	19.0
14a (<i>Z</i>)	7.36	8.46	4.91	5.8	21.2
15a (<i>E</i>)	7.42	8.47	4.90	9.6	19.8
12b (<i>Z</i>)	7.10	8.56	5.10	6.6	20.7
13b (<i>E</i>)	7.24	8.02	4.78	8.5	18.8
14b (<i>Z</i>)	7.09	8.01	4.85	5.7	20.9
15b (<i>E</i>)	7.17	8.04	4.85	9.5	19.8

14b by the 5'-OH. At any rate, these differences may help in distinguishing both stereoisomeric series of analogues. No significant change in the 6'-OH chemical shifts was observed in any of these analogues. The C_{4'} chemical shifts C_{4'}(*Z*) > C_{4'}(*E*) then followed the trend seen in other methylenecyclopropane analogues.¹⁷ Needless to say, all these patterns were also reflected in the corresponding enantiomeric series 4'*R*,5'*R* and 4'*R*,5'*S*.

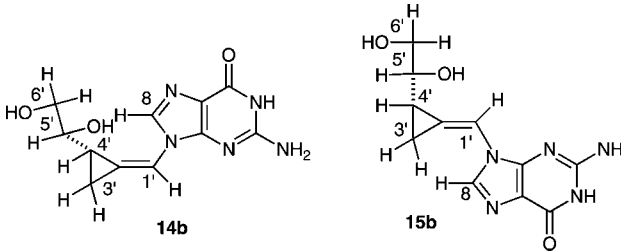
The assignment of the *Z*- and *E*-isomers was unequivocally confirmed by the NOE results with guanine analogues **12b**, **13b**, **14b**, and **15b** (Tables 3 and 4). As expected,¹⁶ the interactions of the *cis*-related protons with the H₈ of purine in an *anti*-like conformation were crucial for assignment. In the *Z*-isomers **12b** and **14b**, strong effects were seen between the H₈ and 5'-OH, H_{6'}(H_{5'}), and H_{4'} complemented by NOE enhancements between the H_{1'} and H_{3'}. By contrast, interactions between the H₈ and H_{3'} as well as between H_{1'} and H_{4'} were typical for the *E*-isomers **12b** and **15b**. In the case of **15b**, the long-range effects between the H_{1'} and H_{5'}(H_{6'}) and even 6'-OH assisted possibly by the rigidity of methylenecyclopropane scaffold were also observed.

The final confirmation of absolute configuration and *Z*,*E*-isomeric structure came from X-ray diffraction of a single crystal of diacetate **34** derived from adenine analogue *ent*-**12a** (Figure 1). The X-ray confirmed the *Z*-configuration of **34** as well as the *anti*-like conformation of the purine base. Given the fact that diacetate **34** does not contain any heavy atom and molybdenum tube was used in the experiment, determination of only relative configuration at the C-4' and C-5' (C-9 and C-10 in Figure 1) was possible. Nevertheless, as indicated above, the configuration at the C-4' is *R* by virtue

Table 3. NOE Data of the *Z*- and *E*-Isomers **12b** and **13b** (DMSO-*d*₆, 300 MHz)


Chemical structures of 12b and 13b are shown above the table. 12b is the Z-isomer and 13b is the E-isomer of a guanine derivative with a methylenecyclopropyl group.

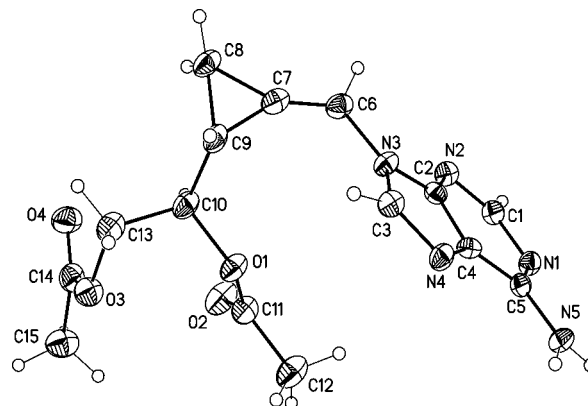
compd	H _{irr}	δ, ppm	H _{obs}	δ, ppm	NOE, %
12b	H ₈	8.56	5'-OH	5.10	2.07
	H ₈	8.56	H _{6'} (H _{5'})	3.39–3.46	4.18
	H ₈	8.56	H _{6'} (H _{5'})	3.10	1.33
	H ₈	8.56	H _{4'}	1.90	3.61
	5'-OH	5.10	H ₈	8.56	3.29
	H _{6'} (H _{5'})	3.39–3.46	H ₈	8.56	0.72
	H _{6'} (H _{5'})	3.10	H ₈	8.56	1.38
	H _{4'}	1.90	H ₈	8.56	3.50
	H _{3'}	1.42	H _{1'}	7.10	1.59
	H ₈	8.02	H _{3'}	1.36–1.40	0.56
13b	H ₈	8.02	H _{3'}	1.61	1.01
	H _{4'}	1.79–1.84	H _{1'}	7.24	0.88
	H _{3'}	1.36–1.42	H ₈	8.02	1.47
	H _{3'}	1.61	H ₈	8.02	2.77
	H _{1'}	7.24	H _{4'}	1.79–1.84	1.67

Table 4. NOE Data of the *Z*- and *E*-Isomers **14b** and **15b** (DMSO-*d*₆, 300 MHz)


Chemical structures of 14b and 15b are shown above the table. 14b is the Z-isomer and 15b is the E-isomer of a guanine derivative with a methylenecyclopropyl group.

compd	H _{irr}	δ, ppm	H _{obs}	δ, ppm	NOE, %
14b	H ₈	8.01	5'-OH	4.84	0.17
	H ₈	8.01	6'-OH	4.64	0.18
	H ₈	8.01	H _{6'} (H _{5'})	3.51	2.33
	H ₈	8.01	H _{4'}	2.05–2.09	1.62
	5'-OH	4.84	H ₈	8.01	0.71
	6'-OH	4.64	H ₈	8.01	4.33
	H _{5'} (H _{6'})	3.51	H ₈	8.01	5.05
	H _{5'} (H _{6'})	3.25–3.31	H ₈	8.01	5.84
	H _{4'}	2.05–2.09	H ₈	8.01	3.45
	H _{3'}	1.30–1.38	H _{1'}	7.09	1.41
	H _{1'}	7.09	H _{3'}	1.30–1.38	1.65
15b	H ₈	8.04	H _{3'}	1.64	1.07
	H ₈	8.04	H _{3'}	1.35–1.40	0.82
	6'-OH	4.63	H _{1'}	7.17	2.05
	H _{5'} (H _{6'})	3.37–3.46	H _{1'}	7.17	1.27
	H _{5'} (H _{6'})	2.97–3.03	H _{1'}	7.17	2.62
	H _{4'}	1.72–1.77	H _{1'}	7.17	0.74
	H _{3'}	1.64	H ₈	8.04	1.48
	H _{3'}	1.30–1.38	H ₈	8.04	2.30

of the starting (*R*)-(–)-(methylenecyclopropyl)methanol (*ent*-**16**) used for the synthesis of analogue *ent*-**12a** and diacetate **34**. The absolute configuration at carbon C-5' is then *R*, thus confirming the ¹H NMR spectroscopic assignment based on intermediates **20**, **21**, and respective enantiomers *ent*-**20**, *ent*-**21** (see above). This established not only the 4'*R*,5'*R* configuration and structure of the *E*-isomer *ent*-**13a** but also of the opposite 4'*S*,5'*S* enantiomers **12a** and **13a**. Stereoisomers

**Figure 1.** ORTEP-3 view of crystal structure of (4'*R*,5'*R*)-diacetate **34** molecule showing 50% probability displacement ellipsoids.**Table 5.** Inhibition of HCMV and EBV Replication by Stereoisomeric 1,2-(Dihydroxyethyl)methylenecyclopropane Analogues of Nucleosides

compd	configuration	EC ₅₀ /CC ₅₀ (μM/μM)		
		HCMV/HFF		
		Towne ^{a,b}	AD169 ^{c,d}	EBV/Akata ^e
12a	Z,4' <i>S</i> ,5' <i>S</i>	>100/>100	300/>300	>20/50.5
13a	E,4' <i>S</i> ,5' <i>S</i>	>100/>100	>60/>300	>20/57
14a	Z,4' <i>S</i> ,5' <i>R</i>	>100/>100	>60/>300	43/55
15a	E,4' <i>S</i> ,5' <i>R</i>	>100/>100	>300/>300	50.4/50.6
12b	Z,4' <i>S</i> ,5' <i>S</i>	>100/>100	>300/>300	43/54
13b	E,4' <i>S</i> ,5' <i>S</i>	>100/>100	>60/>300	>20/67
14b	Z,4' <i>S</i> ,5' <i>R</i>	>100/>100	>300/>300	>20/56
15b	E,4' <i>S</i> ,5' <i>R</i>	>100/>100	>300/>300	18/61
<i>ent</i> - 12a	Z,4' <i>R</i> ,5' <i>R</i>	>100/>100	>60/194	52.6/61.5
<i>ent</i> - 13a	E,4' <i>R</i> ,5' <i>R</i>	>100/>100	>60/264	17/73
<i>ent</i> - 14a	Z,4' <i>R</i> ,5' <i>S</i>	>100/>100	>60/206	60/93.5
<i>ent</i> - 15a	E,4' <i>R</i> ,5' <i>S</i>	>100/>100	>60/194	9.8/72.7
<i>ent</i> - 12b	Z,4' <i>R</i> ,5' <i>R</i>	6.8/>100 ^f	7.5/299 ^{a,g}	8/78
<i>ent</i> - 13b	Z,4' <i>R</i> ,5' <i>R</i>	>100/>100	>60/298	40/75
<i>ent</i> - 14b	Z,4' <i>R</i> ,5' <i>S</i>	>100/>300	>300/>300	>20/62.6
<i>ent</i> - 15b	E,4' <i>R</i> ,5' <i>S</i>	>100/>300	>300/>300	58.7/86.6
3b		0.46/>100 ^h	0.49/>100 ^h	0.22/46 ⁱ
control		1.2–2.4/>100 ^j	0.04–1.5/>100 ^j	3.3/>100 ^k

^a Plaque reduction assay in HFF cells. ^b Visual cytotoxicity in uninfected HFFs used in plaque reduction assays. ^c Cytopathic effect (CPE) inhibition assay. ^d Cytotoxicity by neutral red uptake. ^e DNA hybridization assay. ^f Average of three experiments. ^g EC₅₀ of 11.3 μM by plaque reduction assay in MCMV/MEF. EC₅₀ of ganciclovir was 5.5 μM. ^h Reference 2. ⁱ Reference 28. ^j Ganciclovir. ^k Acyclovir.

mers **14a**, **15a** and *ent*-**14a**, *ent*-**15a** must then have the 4'*S*,5'*R* and 4'*R*,5'*S* configurations, respectively.

Biological Activity

A. Antiviral Effects. Analogues **12a**–**15a**, **12b**–**15b**, and enantiomers *ent*-**12a** to *ent*-**15a**, *ent*-**12b** to *ent*-**15b** were tested for antiviral activity against the following viruses in vitro: herpes simplex viruses 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), hepatitis B and C virus (HBV, HCV). The results for HCMV and EBV are summarized in Table 5. Activity against HCMV was found only for the 4'*R*,5'*R* guanine *ent*-**12b**. It was effective in plaque reduction assay against Towne and AD169 strain of HCMV with EC₅₀ of 6.8 and 7.5 μM, respectively. It was also active in murine cytomegalovirus (MCMV) assay (EC₅₀ of 11.5 μM). It is not cytotoxic, and its efficacy is somewhat lower than that of ganciclovir. The fact that the new active anti-HCMV agent *ent*-**12b** is the *Z*-isomer is not surprising because the *Z*-guanine analogues **1b** and **3b** were highly effective.¹ None of the *E*-isomers of the present

analogues were effective against HCMV, which is in line with the previous results with other methylenecyclopropane analogues¹ such as **2** and **4**. More intriguing is a lack of HCMV potency of adenine analogues **12a–15a** and *ent*-**12a** to *ent*-**15a** because analogues **1a** and **3a** were effective against HCMV.¹ The very narrow range of potent anti-HCMV activity limited to a single compound *ent*-**12b** with unique 4′*R*,5′*R* stereochemistry from a total of eight *Z*-isomeric analogues is a hallmark of this series. The rigidity of methylenecyclopropane system coupled with two centers of chirality is probably responsible for this marked stereoselectivity. Interestingly, an opposite trend, decreased enantioselectivity, was observed in less rigid dihydroxyanalogue **11** with only a single center of asymmetry where both enantiomers were active against HCMV.⁹

Analogue *ent*-**12b** was also the most effective against EBV in Akata cell culture with EC₅₀ of 8 μM (Table 5). It is somewhat less potent than acyclovir (**9**). The other less potent analogues included the *E*-isomers *ent*-**15a**, *ent*-**13a**, and **15b** with EC₅₀ of 9.8–18 μM indicating that the 4′*R*,5′*R* stereochemistry and *Z/E* isomerism are less important for anti-EBV than an anti-HCMV effect. It is noted that effectivity of the *E*-isomers of methylenecyclopropane analogues against EBV was observed before in several instances.^{1,18} Interestingly, the patterns of anti-HCMV and anti-EBV effects of analogue *ent*-**12b** parallel those of cyclopropavir **3b**, although the former is a weaker antiviral. Compound *ent*-**12b** also exhibited a moderate effect against HSV-1 (EC₅₀/CC₅₀ of 50/>100 μM) in BSC-1 cells (ELISA), but it was inactive against HSV-1 and HSV-2 in human foreskin fibroblast (HFF) culture. It was also somewhat effective in VZV/HFF assay (EC₅₀/CC₅₀ of 47/284 μM). None of the analogues was active against hepatitis B or C virus. It is noted that somewhat similar cyclopropane analogue **35** (*n* = 0, B = Gua) described in the patent literature¹⁹ as a mixture of diastereoisomers was without significant activity against HSV-1. The 1′*S*,2′*R*,4′*S* stereoisomers **35** (*n* = 0 or 1, B = Thy, Ura, Ade, or Gua) were also devoid of antiviral activity.^{20,21} Nevertheless, it is emphasized that HCMV and EBV assays with **35** have not been reported.

Regarding the mechanism of antiviral action of active 1,2-dihydroxyethyl analogues, we hypothesize that the phosphorylation cascade generally applicable for activation of nucleosides (including methylenecyclopropane analogues¹), phosphate → diphosphate → triphosphate, is also operative here. Because none of the compounds is active against HSV-1 or HSV-2, viral thymidine kinase likely seems unable to effect the first phosphorylation in contrast to ganciclovir analogue **11** where the hydroxymethyl group is phosphorylated by this enzyme.¹⁰ There is evidence that methylenecyclopropanes **1b**, **2b**, and **3b** are phosphorylated by the action^{3,22–25} of HCMV-encoded phosphotransferase pUL97, and this appears to be the case with *ent*-**12b** as well. Comparison of the activity of *ent*-**12b** to that of cyclopropavir (**3b**) and ganciclovir (**10**) in two HCMV strains with mutated UL97 genes showed that all three compounds were significantly less active against HCMV with UL97 mutations^{23,25} than to wild-type virus (Table 6). Together with the known substrate specificity²⁶ of pUL97 for ganciclovir (**10**), we take this as a strong evidence that this enzyme phosphorylates *ent*-**12b**.

B. Adenosine Deaminase (ADA). Adenine analogues **12a–15a** and *ent*-**12a** to *ent*-**15a** were tested for substrate activity toward ADA (Table 7). All active analogues are only moderate substrates for the enzyme. As observed in the previous cases of methylenecyclopropane analogues, the *E*-isomers are more reactive than the *Z*-isomers. This effect does not depend

Table 6. Activity of *ent*-**12b** against Drug Resistant HCMV

compd	EC ₅₀ (μM) ^a in virus strain		
	Towne ^b	2696r ^c	E8 ^d
<i>ent</i> - 12b	8	>100	72
cyclopropavir (3b)	0.6	28	8
ganciclovir (10)	2	42	22

^a Data from a plaque reduction assay with four drug concentrations in duplicate. ^b Wild-type virus from which isolates 2696r and E8 were obtained.

^c Virus isolated for resistance to cyclopropavir (**3b**) that has a truncated UL97 gene. ^d Virus with two point mutations introduced into gene UL97.

Table 7. Deamination of Adenine 1,2-(Dihydroxyethyl)methylenecyclopropane Analogues of Nucleosides by Adenosine Deaminase^a

compd	isomer	configuration	deamination, %	
			24 h	48 h
12a	<i>Z</i>	4′ <i>S</i> ,5′ <i>S</i>	45	80
13a	<i>E</i>	4′ <i>S</i> ,5′ <i>S</i>	85	100
14a	<i>Z</i>	4′ <i>S</i> ,5′ <i>R</i>	65	80
15a	<i>E</i>	4′ <i>S</i> ,5′ <i>R</i>	100	100
<i>ent</i> - 12a	<i>Z</i>	4′ <i>R</i> ,4′ <i>R</i>	0	0
<i>ent</i> - 13a	<i>E</i>	4′ <i>R</i> ,4′ <i>R</i>	100	100
<i>ent</i> - 14a	<i>Z</i>	4′ <i>R</i> ,5′ <i>S</i>	0	0
<i>ent</i> - 15a	<i>E</i>	4′ <i>R</i> ,5′ <i>S</i>	65	85

^a For details, see Experimental Section.

much on the stereochemistry of the side chain. Thus, all *E*-isomers were deaminated from 80% to 100% within 24–48 h, whereas the *Z*-analogues *ent*-**12a** and *ent*-**14a** were resistant to deamination.

Experimental Section

General Methods. The UV spectra were measured in ethanol, and NMR spectra were determined in CD₃SOCD₃ at 400 MHz (¹H) and 100 MHz (¹³C) unless stated otherwise. Mass spectra were determined in electron-impact (EI-MS) or electrospray ionization (ESI-MS, methanol–AcONa) mode. Optical rotations were measured on JASCO digital polarimeter DIP-370. The (*S*)-(+)- and (*R*)-(–)-(methylenecyclopropyl)methanols (**16** and *ent*-**16**) were prepared as described^{11–13} from (*S*)-(+)- and (*R*)-(–)-epichlorohydrin. Enantiomeric epichlorohydrins were obtained as reported.²⁷

They were converted to benzyl (*S*)- and (*R*)-glycidyl ethers²⁷ using a procedure for racemic compounds,²⁸ and enantiomeric enhancements (ee) were determined by chiral HPLC (Chiralcel AD column was used instead of Chiralcel OD²⁷). The ee values of benzyl (*S*)- and (*R*)-glycidyl ether were 96% and 99%, respectively. A characterization of enantiomeric compounds by UV (where applicable), ¹H, ¹³C NMR, and MS is reported for only one set of enantiomers. The data of the opposite series were essentially identical. The prefixes “*ent*–” before compound numbers indicate opposite enantiomers. Optical rotation was determined for all enantiomeric compounds. All biologically tested analogues were ≥95% pure as indicated by C, H, N analyses. The optical purity of all adenine analogues was at least 95% as shown by chiral HPLC (Table 1).

(–)-(R,S)-2-Hydroxy-2-[(S)-2-methylenecyclopropyl]acetone-trile (18). To a solution of oxalyl chloride (7.5 mL, 85.0 mmol) in CH₂Cl₂ (100 mL) at –60 °C was added DMSO (12.5 mL, 175.0 mmol) in CH₂Cl₂ (25 mL) with stirring at –60 °C followed, after 45 min, by a dropwise addition of (*S*)-(+)-(methylenecyclopropyl)methanol (**16**, 4.2 g, 50.0 mmol) in CH₂Cl₂ (25 mL). The stirring was continued for 45 min, whereupon triethylamine (35 mL, 0.25 mol) was added and the reaction mixture containing crude aldehyde **17** was allowed to warm to room temperature. Then 1 M HCl saturated with NaCl (275 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (4 × 50 mL). The organic layers were combined, and a saturated solution of NH₄Cl (30 mL) was added with stirring and external ice-cooling followed by NaCN (3.67 g, 75 mmol). The stirring was continued for 30 min, the layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (6 ×

25 mL). The combined organic phase was dried over MgSO_4 . The solvent was evaporated and the crude product was chromatographed on a silica gel column using hexanes–diethyl ether (10:1 to 1:1) to give nitrile **18** as a colorless oil (2.96 g, 54.3%). $[\alpha]_D^{26} -11.8^\circ$ (*c* 1.0, CHCl_3). ^1H NMR (300 MHz, CDCl_3) δ 1.17–1.27 (m, 1H), 1.43–1.52 (m, 1H), 1.93–2.01 (m, 1H, cyclopropane), 3.90 (bs, 1H, OH), 4.10, 4.29 (2d, *J* = 8.4, 7.2 Hz, 1H, CHO), 5.53–5.55 (m, 1H), 5.61–5.65 (m, 1H, $=\text{CH}_2$). ^{13}C NMR (75 MHz) 7.5, 9.2, 18.9, 19.2 (cyclopropane), 63.4, 63.7 (CHO), 107.00, 107.04 ($=\text{CH}_2$), 118.7, 119.1 ($=\text{C}$), 128.6, 129.5 (CN). EI-HRMS calcd for $\text{C}_6\text{H}_7\text{NO}$ (M) 109.0528, found 109.0529.

(+)-(R,S)-2-Hydroxy-2-[(R)-2-methylenecyclopropyl]acetone (ent-18). The procedure described above was performed with (R)-(-)-(methylenecyclopropyl)methanol (ent-16, 4.8 g, 57 mmol) to give nitrile ent-18 via aldehyde ent-17 (3.55 g, 57%), $[\alpha]_D^{23} 11.4^\circ$ (*c* 1.58, CHCl_3).

(-)-Benzyl (S)-2-Hydroxy-2-[(S)-2-methylenecyclopropyl]acetate (20) and (-)-Benzyl (R)-2-Hydroxy-2-[(S)-2-methylenecyclopropyl]acetate (21). A solution of nitrile **18** (2.76 g, 25.32 mmol) in CH_2Cl_2 (100 mL) was stirred with 37% HCl (25 mL) at room temperature for 2 h. The volatiles were evaporated at 18 torr and room temperature, and a solution of the residue in ether was passed through a 5 cm silica gel pad. The pad was washed with hexanes– Et_2O (1:1, 180 mL), and the filtrate was concentrated to give acid **19** (2.91 g, 90%), which was directly used in the next step.

Benzyl bromide (8.4 mL, 70.3 mmol) was added dropwise with stirring to acid **19** (2.8 g, 21.9 mmol) in DMF (33 mL), anhydrous K_2CO_3 (5.07 g, 36.7 mmol), and NBu_4I (2.60 g, 7.03 mmol) at room temperature. The stirring was continued for 3.5 h, the solvent was evaporated, and the residue was partitioned between water (60 mL) and Et_2O (4 $\times$ 60 mL). The organic phase was washed with 10% HCl (25 mL), 5% NaHCO_3 (25 mL), and brine (20 mL). After the mixture was dried (MgSO_4), the solvent was evaporated and the crude product was chromatographed on a silica gel column in hexanes– Et_2O (10:1 to 5:1) to give the (S,S)-isomer **20** (1.67 g, 35%) followed by (S,R)-isomer **21** (1.81 g, 38%).

(S,S)-Isomer **20**: $[\alpha]_D^{26} -7.3^\circ$ (*c* 1.0, CHCl_3). ^1H NMR (CDCl_3) δ 1.28–1.34 (m, 2H), 1.82–1.85 (m, 1H, cyclopropane), 2.73 (s, 1H, OH), 4.04 (d, 1H, *J* = 5.6 Hz, CHO), 5.23 (AB, 2H, $J_{\text{AB}} = 12$ Hz, CH_2 of Bn), 5.46 (d, 2H, *J* = 2.4 Hz, $=\text{CH}_2$), 7.37 (m, 5H, Ph). ^{13}C NMR 6.8, 18.8 (cyclopropane), 67.5 (CHO), 71.0 (CH_2 of Bn), 105.7 ($=\text{CH}$), 128.5, 128.7, 128.9, 130.9, 135.5 ($=\text{C}$, Ph), 174.3 (C=O). ESI-MS 241.3 (M + Na, 100.0). Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{O}_3 \cdot 0.2\text{H}_2\text{O}$.

(S,R)-Isomer **21**: $[\alpha]_D^{25} -45.3^\circ$ (*c* 1.02, CHCl_3). ^1H NMR (CDCl_3) δ 1.19 (m, 1H), 1.37 (tt, 1H, *J* = 8.8, 2.4 Hz), 1.72–1.79 (m, 1H, cyclopropane), 2.93 (s, 1H, OH), 3.80 (d, 1H, *J* = 7.2 Hz, CHO), 5.20 (AB, 2H, $J_{\text{AB}} = 12$ Hz, CH_2 of benzyl), 5.40, (s, 1H), 5.43 (d, 1H, *J* = 1.6 Hz, $=\text{CH}_2$), 7.37 (m, 5H, Ph). ^{13}C NMR 8.0, 19.4 (cyclopropane), 67.8 (CHO), 72.6 (CH_2 of Bn), 106.0 ($=\text{CH}_2$), 128.6, 128.8, 128.9, 130.9, 135.4 ($=\text{C}$, Ph), 174.2 (C=O). ESI-MS 241.3 (M + Na, 100.0). Anal. ($\text{C}_{13}\text{H}_{14}\text{O}_3 \times 0.3 \text{H}_2\text{O}$) C, H.

(+)-Benzyl (R)-2-Hydroxy-2-[(R)-2-methylenecyclopropyl]acetate (ent-20) and (+)-Benzyl (S)-2-Hydroxy-2-[(R)-2-methylenecyclopropyl]acetate (ent-21). The protocol described above was repeated with nitrile ent-18 (3.10 g, 28 mmol) to give esters ent-20 (1.71 g, 28%) and ent-21 (2.52 g, 41%) via acid ent-19 as an intermediate. ent-20: $[\alpha]_D^{26} 6.3^\circ$ (*c* 1.04, CHCl_3). ent-21: $[\alpha]_D^{26} 44.9^\circ$ (*c* 1.02, CHCl_3).

(-)-(S)-1-[(S)-2-Methylenecyclopropyl]ethane-1,2-diol (22). DIBALH in hexanes (1M, 24 mL, 24 mmol) was added to a solution of the (S,S)-ester **20** (1.50 g, 6.9 mmol) in THF (40 mL) with stirring at 0 $^\circ\text{C}$ during 10 min under N_2 . The stirring was continued for 1 h. The reaction was quenched by a dropwise addition of HCl (5%, 35 mL). After stirring for 2 h, it was extracted with Et_2O (15 $\times$ 30 mL). The organic phase was washed successively with saturated NaHCO_3 (2 $\times$ 30 mL) and brine (2 $\times$ 30 mL). After drying (MgSO_4), the solvents were evaporated and the crude product was chromatographed on a silica gel column in hexanes– Et_2O (2:1 to 1:3) to give diol **22** (580 mg, 74%) as a colorless oil, $[\alpha]_D^{24} -3.5^\circ$

(*c* 1.03, CHCl_3). ^1H NMR (CDCl_3) δ 0.97–1.01 (m, 1H), 1.27 (tt, 1H, *J* = 8.8, 1.6 Hz), 1.57–1.62 (m, 1H, cyclopropane), 3.10 (bs, 2H, OH), 3.32 (dt, 1H, *J* = 7.6, 3.2 Hz), 3.56 (dd, 1H, *J* = 11.4, 8.2 Hz), 3.73 (dd, 1H, *J* = 11.2, 3.2 Hz, CHO, CH_2O), 5.44, 5.53 (2m, 2H, $=\text{CH}_2$). ^{13}C NMR 7.3, 17.9 (cyclopropane), 66.4 (CH_2O), 74.9 (CHO), 104.7 ($=\text{CH}$), 132.6 ($=\text{CH}_2$). EI-HRMS calcd for $\text{C}_6\text{H}_8\text{O}$ (M – H_2O) 96.0575, found 96.0572. Anal. ($\text{C}_6\text{H}_{10}\text{O}_2 \cdot 0.2\text{H}_2\text{O}$) C, H.

(+)-(R)-1-[(R)-2-Methylenecyclopropyl]ethane-1,2-diol (ent-22). The procedure described above was performed with (R,R)-ester ent-20 (1.65 g, 7.6 mmol) to furnish diol ent-22 (630 mg, 73%). $[\alpha]_D^{25} 2.7^\circ$ (*c* 1.05, CHCl_3).

(+)-(R)-1-[(S)-2-Methylenecyclopropyl]ethane-1,2-diol (23). The reduction of (R,S)-ester **21** (1.6 g, 7.34 mmol) followed the above procedure to give diol **23** (660 mg, 79%) as a white solid: mp 69–71 $^\circ\text{C}$, $[\alpha]_D^{27} 25.4^\circ$ (*c* 1.0, CHCl_3). ^1H NMR (CDCl_3) δ 1.06–1.10 (m, 1H), 1.34 (tt, 1H, *J* = 8.8, 1.6 Hz), 1.54–1.60 (m, 1H, cyclopropane), 3.01, 3.10 (2bs, 2H, OH), 3.21 (dt, 1H, 8.0, *J* = 2.7 Hz), 3.59 (dd, 1H, *J* = 10.8, 7.2 Hz), 3.69 (poorly resolved dd, 1H, *J* = 10.4 Hz, CHO, CH_2O), 5.40 (d, 1H, *J* = 2.4 Hz), 5.42 (d, 1H, *J* = 1.6 Hz, $=\text{CH}_2$). ^{13}C NMR 8.3, 18.5 (cyclopropane), 66.7 (CH_2O), 75.2 (CHO), 104.8 ($=\text{CH}$), 132.0 ($=\text{CH}_2$). EI-HRMS calcd for $\text{C}_6\text{H}_8\text{O}$ (M – H_2O) 96.0575, found 96.0580. Anal. ($\text{C}_6\text{H}_{10}\text{O}_2 \cdot 0.45\text{H}_2\text{O}$) C, H.

(-)-(S)-1-[(R)-2-Methylenecyclopropyl]ethane-1,2-diol (ent-23). The reduction of (S,R)-ester ent-21 (2.0 g, 9.17 mmol) followed the above procedure to give diol ent-23 (805 mg, 77%): mp 73–74 $^\circ\text{C}$, $[\alpha]_D^{26} -25.0^\circ$ (*c* 1.05, CHCl_3).

(+)-(S)-1-[(S)-2-Methylenecyclopropyl]ethane-1,2-diyl Diacetate (24). Acetic anhydride (3 mL) was added dropwise to a stirred solution of diol **22** (520 mg, 4.56 mmol) in pyridine (1.5 mL) at room temperature. The stirring was continued for 10 h, the reaction was quenched with water, and product was extracted with ice cold Et_2O (100 mL). The combined organic phase was washed successively with saturated aqueous CuSO_4 , 5% HCl (4 $\times$ 25 mL), aqueous NaHCO_3 (3 $\times$ 20 mL), and brine (3 $\times$ 20 mL). It was dried (MgSO_4), the solvent was evaporated, and the residue was chromatographed on a silica gel column (hexanes– Et_2O , 50:1 to 10:1) to give diacetate **24** (760 mg, 84%) as a colorless oil: $[\alpha]_D^{24} 80.9^\circ$ (*c* 0.97, CHCl_3). ^1H NMR (CDCl_3) δ 1.06–1.09 (m, 1H), 1.37 (tt, 1H, *J* = 9.2, 1.6 Hz), 1.71 (m, 1H, cyclopropane), 2.05, 2.07 (2s, 6H, CH_3), 4.09 (dd, 1H, *J* = 12.0, 7.2 Hz), 4.34 (dd, 1H, *J* = 12.4, 2.8 Hz), 4.71 (dt, 1H, *J* = 8.8, 3.2 Hz, CHO, CH_2O), 5.42, 5.44 (2s, 2H, $=\text{CH}_2$). ^{13}C NMR 7.9, 15.8 (cyclopropane), 21.0, 21.3 (CH_3), 65.0 (CH_2O), 73.8 (CHO), 105.2 ($=\text{CH}$), 131.2 ($=\text{C}$), 170.5, 171.0 (C=O). ESI-MS 221 (M + Na, 100.0). EI-HRMS calcd for $\text{C}_{10}\text{H}_{15}\text{O}_4$ 199.0970, found 199.0966. Anal. ($\text{C}_{10}\text{H}_{14}\text{O}_4$) C, H.

(-)-(R)-1-[(R)-2-Methylenecyclopropyl]ethane-1,2-diyl Diacetate (ent-24). The protocol described above was followed with diol ent-22 (600 mg, 5.26 mmol) to give diacetate ent-24 (840 mg, 81%), $[\alpha]_D^{27} -78.1^\circ$ (*c* 1.0, CHCl_3).

(+)-(R)-1-[(S)-2-Methylenecyclopropyl]ethane-1,2-diyl Diacetate (25). The procedure described above was performed with diol **23** (610 mg, 5.35 mmol) to give diacetate **25** (910 mg, 86%) as a colorless oil: $[\alpha]_D^{26} 31.6^\circ$ (*c* 1.0, CHCl_3). ^1H NMR (CDCl_3) δ 1.10–1.14 (m, 1H), 1.28 (tt, 1H, *J* = 8.8, 2.4 Hz), 1.58–1.63 (m, 1H, cyclopropane), 1.99, 2.00 (2s, 6H, CH_3), 4.08–4.19 (m, 2H), 4.53–4.68 (m, 1H, CH_2O , CHO), 5.40 (m, 2H, $=\text{CH}_2$). ^{13}C NMR 8.8, 16.5 (cyclopropane), 20.9, 21.2 (CH_3), 65.4 (CH_2O), 73.6 (CHO), 105.6 ($=\text{CH}$), 131.0 ($=\text{C}$), 170.6, 170.7 (C=O). ESI-MS 221 (M + Na, 100.0). Anal. ($\text{C}_{10}\text{H}_{14}\text{O}_4$) C, H.

(-)-(S)-1-[(R)-2-Methylenecyclopropyl]ethane-1,2-diyl Diacetate (ent-25). The protocol described above was followed with diol ent-23 (720 mg, 6.32 mmol) to give diacetate ent-25 (1.04 g, 81%), $[\alpha]_D^{27} -33.9^\circ$ (*c* 0.98, CHCl_3).

(S)-1-[(1R,2S)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate + (S)-1-[(1R,2R)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate (26). Pyridinium tribromide (1.64 g, 5.15 mmol) was added with stirring to a solution of diacetate **24** (680 mg, 3.43 mmol) in CH_2Cl_2 (18 mL) at –20 $^\circ\text{C}$.

The reaction mixture was allowed to warm to room temperature. After 12 h, it was diluted with AcOEt (100 mL), and the resultant solution was washed sequentially with saturated $\text{Na}_2\text{S}_2\text{O}_3$ (2×25 mL), NaHCO_3 (2×20 mL), and water (20 mL). The organic phase was dried over MgSO_4 , and the solvents were evaporated. The crude product was chromatographed on a silica gel column in hexanes– Et_2O (20:1 to 5:1) to afford *cis,trans*-dibromide **26** (1.06 g, 86%) as a colorless oil: $[\alpha]_D^{25}$ 46.5° (*c* 1.1, CHCl_3). ^1H NMR (CDCl_3) δ 1.00 (t, $J = 6.4$ Hz), 1.22–1.31 (m), 1.34–1.43 (m), 1.60–1.64 (m, 3H, cyclopropane), 2.06, 2.07, 2.09, 2.10 (4s, 6H, CH_3), 3.64–3.80 (m, 2H, CH_2Br), 4.03–4.14 (m), 4.35–4.46 (m, 2H), 4.68–4.73 (m), 4.81–4.85 m, 1H, CH_2O , CHO). ^{13}C NMR 20.9, 21.0, 21.2, 21.3, 22.3, 22.6, 27.1, 32.0, 35.5, 38.0, 40.2, 43.4, 64.6, 64.7, 70.8, 74.7, 170.1, 170.4, 170.8, 170.9. ESI-MS ($\text{MeOH} + \text{AcOK}$) 395, 397, 399 ($\text{M} + \text{K}$, 48.8, 100.0, 55.6).

(R)-1-[(1S,2R)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate + (R)-1-[(1S,2S)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate (ent-26). The reaction was performed as described above with diacetate *ent*-24 (740 mg, 3.74 mmol) to give *cis,trans*-dibromide *ent*-26 (1.14 g, 85%): $[\alpha]_D^{25}$ –52.8° (*c* 1.05, CHCl_3).

(R)-1-[(1R,2S)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate + (R)-1-[(1R,2R)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate (27). The protocol described above was performed with diacetate **25** (810 mg, 4.09 mmol) to give the *cis,trans*-dibromide **27** (1.11 g, 76%) as a colorless oil: $[\alpha]_D^{27}$ 11.6° (*c* 1.0, CHCl_3). ^1H NMR (CDCl_3) δ 1.12 (t, $J = 7.5$ Hz), 1.15–1.22 (m), 1.31–1.36 (m), 1.41–4.53 (m), 1.83–1.90 (m, 3H, cyclopropane), 2.005, 2.009, 2.02 (3s, 6, CH_3), 3.46, 3.74 (AB, $J_{\text{AB}} = 11.2$ Hz), 3.70, 3.85 (AB, 2H, $J_{\text{AB}} = 12.4$ Hz, CH_2Br), 4.17–4.25 (m), 4.36–4.40 (2d, $J = 3.2, 4.4$ Hz, 2H), 4.77–4.82, 4.84–4.89 (2m, 1H, CH_2O , CHO). ^{13}C NMR 20.95, 21.00, 21.17, 21.23, 23.4, 23.7, 26.2, 31.8, 35.8, 37.4, 40.9, 44.0, 64.5, 65.6, 69.8, 74.6, 170.3, 170.6, 170.7. ESI-MS ($\text{MeOH} + \text{AcOK}$) 395, 397, 399 ($\text{M} + \text{K}$, 50.0, 100.0, 56.9).

(R)-1-[(1S,2R)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate + (S)-1-[(1S,2S)-2-Bromo-2-(bromomethyl)cyclopropyl]ethane-1,2-diyl Diacetate (ent-27). The reaction was performed as described above with diacetate *ent*-25 (740 mg, 3.74 mmol) to give *cis,trans*-dibromide *ent*-27 (1.14 g, 85%): $[\alpha]_D^{25}$ –8.9° (*c* 1.0, CHCl_3).

9-[(Z)-(S)-2-[(S)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine + 9-[(E)-(S)-2-[(S)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine (28a). A mixture of adenine (200 mg, 1.48 mmol), dibromide **26** (490 mg, 1.37 mmol), and K_2CO_3 (1.13 g, 8.2 mmol) in DMF (7 mL) was stirred under N_2 at 100–105 °C for 7 h. After the mixture was cooled, the solids were filtered off using a silica gel pad that was washed with DMF (60 mL). The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel column in AcOEt– MeOH (50:1 to 20:1) to give the *Z,E*-isomers **28a** (254 mg, 56%): mp 141–145 °C, $[\alpha]_D^{22}$ 65.8° (*c* 1.06, MeOH). UV λ_{max} 224 nm (ϵ 25 700), 262 (ϵ 12 800), 279 (ϵ 9100). ^1H NMR (CDCl_3) δ 1.43–1.47 (m), 1.52–1.56 (m), 1.70, 1.81 (dt, 1H, $J = 8.8, 2.4$ Hz), 2.00–2.03 (m, partially overlapped with CH_3 at 2.06), 2.25–2.32 (m, 3H, cyclopropane), 1.91, 2.06, 2.09, and 2.11 (4s, 6H, CH_3), 4.15–4.19 (m), 4.41, 4.44 (dt, 2H, $J = 12.0, 1.6$ Hz, H_6), 4.64–4.69 (m), 4.77–4.81 (m, 1H, H_5), 6.32, 6.40 (2bs, 2H, NH_2), 7.49, 7.56 (m, 1H, H_1), 8.25, 8.36, 8.37, 8.40 (4s, 2H, H_2, H_8). ^{13}C NMR 7.6, 9.3, 15.9, 17.6, 20.99, 21.03, 21.3, 64.6, 64.8, 73.0, 74.4, 112.0, 112.1, 112.9, 113.6, 119.3, 119.4, 137.5, 137.9, 148.9, 152.7, 152.9, 155.4, 170.0, 170.4, 170.87, 170.92. ESI-MS 332 ($\text{M} + \text{H}$, 44.5), 354 ($\text{M} + \text{Na}$, 100.0), 685 (2M + Na, 85.8).

9-[(Z)-(R)-2-[(R)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine + 9-[(E)-(R)-2-[(R)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine (ent-28a). A mixture of adenine (230 mg, 1.7 mmol), dibromide *ent*-26 (580 mg, 1.62 mmol), and K_2CO_3 (1.34 g, 9.7 mmol) in DMF (8 mL) was stirred under N_2 for 48 h at room temperature and then for 8 h at 100–105 °C. The workup followed the protocol described above to give the *Z,E*-isomers *ent*-28a (327 mg, 61%): mp 163–165 °C, $[\alpha]_D^{22}$ –75.5° (*c* 1.0, MeOH).

9-[(Z)-(S)-2-[(Z)-(R)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine + 9-[(E)-(S)-2-[(R)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine (29a). The reaction of adenine with dibromide **27** was performed on the same scale as described for compound **28a** (reaction time was 12 h at room temperature and 10 h at 100–105 °C) to give the *Z,E*-isomers **29a** (273 mg, 58%): mp 135–139 °C, $[\alpha]_D^{22}$ 27.8° (*c* 1.0, MeOH). UV λ_{max} 226 nm (ϵ 26 500), 263 (ϵ 12 700), 278 (ϵ 9400). ^1H NMR (CDCl_3) δ 1.51–1.55 (m), 1.60 (dt, $J = 9.2, 2.0$ Hz), 1.66–1.70 (m), 1.79 (dt, $J = 8.8, 2.4$ Hz, H_3), 2.00, 2.01, 2.10, 2.11 (4s, 6H, CH_3), 2.07–2.10 (m, partly overlapped with 2.10), 2.18–2.23 (m, 1H, H_4), 4.24, 4.01 and 4.23, 4.03 (2AB, $J_{\text{AB}} = 12.2$ Hz, 2H, H_6), 4.72, 5.21 (2s, 1H, H_5), 6.28, 6.34 (2bs, 2H, NH_2), 7.49, 7.61 (2d, $J = 1.6$ Hz, 1H, H_1), 8.23, 8.24, 8.37 (3s, 2H, H_2, H_8). ^{13}C NMR 10.62, 10.5, 16.6, 18.3, 20.9, 20.96, 21.02, 64.6, 65.1, 70.1, 73.0, 112.51, 112.54, 113.1, 114.3, 119.3, 119.4, 137.6, 138.8, 148.9, 151.8, 152.2, 154.9, 155.02, 170.1, 170.6, 170.7, 170.8. ESI-MS 332 ($\text{M} + \text{H}$, 38.9), 354 ($\text{M} + \text{Na}$, 100.0), 685 (2M + Na, 56.5).

9-[(Z)-(R)-2-[(S)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine + 9-[(E)-(R)-2-[(S)-1,2-Diacetoxyethyl]cyclopropylidene]methyladenine (ent-29a). A mixture of adenine (220 mg, 1.63 mmol), dibromide *ent*-27 (550 mg, 1.54 mmol), and K_2CO_3 (1.28 g, 9.3 mmol) in DMF (8 mL) was stirred under N_2 for 24 h at room temperature and 8 h at 100–105 °C. The workup followed the above procedure to furnish the *Z,E*-isomers *ent*-29a (280 mg, 55%): mp 152–156 °C, $[\alpha]_D^{22}$ –36.6° (*c* 1.0, MeOH).

2-Amino-6-chloro-9-[(Z)-(S)-2-[(S)-1,2-diacetoxyethyl]cyclopropylidene]methylpurine + 2-Amino-6-chloro-9-[(E)-(S)-2-[(S)-1,2-diacetoxyethyl]cyclopropylidene]methylpurine (28b). The reaction was performed as described for adenine *Z,E*-isomers **28a** with 2-amino-6-chloropurine (270 mg, 1.6 mmol), dibromide **26** (550 mg, 1.54 mmol), and K_2CO_3 (1.27 g, 9.2 mmol) in DMF (8 mL). Reaction time was 24 h at room temperature and 6 h at 100–105 °C. The crude product was chromatographed in hexanes–AcOEt (1:1 to 1:2) to give the *E,Z*-isomers **28b** (320 mg, 57%): mp 142–147 °C, $[\alpha]_D^{26}$ 80.0° (*c* 1.18, CHCl_3). UV λ_{max} 311 nm (ϵ 7800), 228 (ϵ 29 000). ^1H NMR (CDCl_3) δ 1.43–1.47, 1.51–1.55 (2m), 1.69, 1.81 (2dt, 1H, $J = 8.8, 1.6$ Hz, H_3), 2.25–2.31 (m, 1H, H_4), 1.96, 2.06, 2.09, 2.12 (4s, 6H, CH_3), 4.14–4.20, 4.34–4.44 (2m, 2H, H_6), 4.71–4.75, 4.78–4.82 (2m, 1H, H_5), 5.36 (bs, 2H, NH_2), 7.31, 7.38 (2 poorly resolved d, 1H, H_1), 8.17, 8.29 (2s, 1H, H_8). ^{13}C NMR 7.5, 9.2, 16.0, 17.9, 20.9, 21.0, 21.1, 21.2, 64.5, 64.8, 72.9, 74.3, 111.7, 111.8, 113.1, 113.6, 125.1, 125.2, 138.9, 139.5, 151.6, 151.7, 152.5, 159.50, 159.51, 170.3, 170.4, 170.85, 170.89. ESI-MS 366, 368 ($\text{M} + \text{H}$, 22.2, 7.4), 388, 390 ($\text{M} + \text{Na}$, 100.0, 30.0).

2-Amino-6-chloro-9-[(Z)-(R)-2-[(R)-1,2-diacetoxyethyl]cyclopropylidene]methylpurine + 2-Amino-6-chloro-9-[(E)-(R)-2-[(R)-1,2-diacetoxyethyl]cyclopropylidene]methylpurine (ent-28b). A reaction of 2-amino-6-chloropurine (350 mg, 2.07 mmol) with dibromide *ent*-26 (720 mg, 2.0 mmol) and K_2CO_3 was performed as indicated above to give the *E,Z*-isomers *ent*-28b (360 mg, 46%): mp 136–140 °C, $[\alpha]_D^{26}$ –87.5° (*c* 1.01, CHCl_3).

2-Amino-6-chloro-9-[(Z)-(S)-2-[(R)-1,2-diacetoxyethyl]cyclopropylidene]methylpurine + 2-Amino-6-chloro-9-[(E)-(S)-2-[(R)-1,2-diacetoxyethyl]cyclopropylidene]methylpurine (29b). The protocol described above was followed with 2-amino-6-chloropurine (310 mg, 1.83 mmol), dibromide **27** (620 mg, 1.73 mmol), and K_2CO_3 (1.43 g, 10.4 mmol) at room temperature for 4 h and at 100–105 °C for 7 h. After chromatography in hexanes–AcOEt (1:1 to 1:3), the *E,Z*-isomers **29b** were obtained (348 mg, 55%): mp 153–156 °C, $[\alpha]_D^{27}$ 44.7° (*c* 1.1, CHCl_3). UV λ_{max} 311 nm (ϵ 8000), 228 (ϵ 35 400). ^1H NMR (CDCl_3) δ 1.50–1.60 (m), 1.67–1.68 (m), 1.78 (t, $J = 8.9$ Hz, 3H, cyclopropane), 2.06–2.11 (m, partially overlapped with 2.04), 2.35–2.41 (2m, 3H, cyclopropane), 2.00, 2.03, 2.10, 2.12 (4s, 6H, CH_3), 3.96–4.01 (dd, $J = 12.0, 6.2$ Hz), 4.24–4.30 (m), 4.73–4.76 (m, 3H, H_6, H_5), 5.27, 5.26 (2bs, 2H, NH_2), 7.44, 7.32 (2bs, 1H, $J = 1.6$ Hz, H_1), 8.18, 8.19 (2s, 1H, H_8). ^{13}C NMR 6.2, 10.4, 16.5, 18.4, 20.91, 21.0, 21.2, 64.3, 65.2, 70.3, 72.9, 112.3, 112.6, 114.0, 125.25, 125.27, 138.8, 140.0, 151.8, 152.6, 152.7, 159.57, 159.61,

170.2, 170.6, 170.7, 170.9. ESI-MS 366, 368 (M + H, 26.0, 8.3), 388, 390 (M + Na, 100.0, 32.3).

2-Amino-6-chloro-9-((Z)-(R)-2-[(S)-1,2-diacetoxyethyl]cyclopropylidene)methyl-purine + 2-Amino-6-chloro-9-((E)-(R)-2-[(S)-1,2-diacetoxyethyl]cyclopropylidene)methyl-purine (ent-29b). The reaction was performed as described above with dibromide *ent-27*. Reaction time was 24 h at room temperature and 7 h at 100–105 °C: yield 340 mg (53%) of the *E,Z*-isomers *ent-29b*, mp 166–169 °C, $[\alpha]_D^{27} -47.0^\circ$ (*c* 1.01, CHCl₃).

(+)-9-((Z)-(S)-2-[(S)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (12a) and (-)-9-((E)-(S)-2-[(S)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (13a). A mixture of the *Z,E*-isomers **28a** (240 mg, 0.72 mmol) and K₂CO₃ (200 mg, 1.45 mmol) in methanol (15 mL) was stirred for 45 min at room temperature. The solvent was evaporated and the residue was chromatographed on a silica gel column using AcOEt–CH₂Cl₂–MeOH (15:15:1 to 5:5:1) to give the *Z*-isomer **12a** (64 mg, 36%) followed by *E*-isomer **13a** (88 mg, 49%).

Z-Isomer **12a**: mp 271–272 °C, $[\alpha]_D^{27} 54.6^\circ$ (*c* 1.09, DMSO). UV $\lambda_{\max}$ 278 nm (ϵ 8400), 258 (ϵ 11 900), 229 (ϵ 25 500). ¹H NMR δ 1.27 (poorly resolved t, 1H, H_{4'}), 1.47 (t, 2H, *J* = 8.0 Hz, H_{3'}), 2.00 (poorly resolved dd, 1H, H_{4'}), 3.09–3.15 (m, 1H), 3.40–3.51 (m, 2H, H_{5'}, H_{6'}), 4.67 (t, 1H, *J* = 5.0 Hz, 6'-OH), 5.23 (d, *J* = 4.8 Hz, 5'-OH), 7.31 (bs, 2H, NH₂), 7.38 (s, 1H, H_{1'}), 8.15 (s, 1H, H₂), 8.96 (s, 1H, H₈). ¹³C NMR 6.7 (C_{3'}), 20.8 (C_{4'}), 66.5 (C_{6'}), 75.1 (C_{5'}), 110.8, 115.5, 119.0, 137.8, 148.7, 153.6, 156.7 (adenine, C_{1'}, C_{2'}). ESI-MS 248 (M + H, 95.9), 270 (M + Na, 100.0). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

E-isomer **13a**: mp 245–247 °C, $[\alpha]_D^{27} -3.6^\circ$ (*c* 0.67, DMSO). UV $\lambda_{\max}$ 278 nm (ϵ 8100), 262 (ϵ 11 100), 228 (ϵ 24 700). ¹H NMR δ 1.43–1.46 (m), 1.66 (t, 2H, *J* = 8.8 Hz, H_{3'}), 1.83–1.88 (m, 1H, H_{4'}), 3.17–3.22 (m, 1H), 3.42–3.46 (m, 2H, H_{5'}, H_{6'}), 4.66 (t, 1H, *J* = 4.8 Hz, 6'-OH), 4.83 (d, *J* = 4.0 Hz, 5'-OH), 7.33 (bs, 2H, NH₂), 7.49 (s, 1H, H_{1'}), 8.16 (s, 1H, H₂), 8.46 (s, 1H, H₈). ¹³C NMR 8.7 (C_{3'}), 19.0 (C_{4'}), 66.3 (C_{6'}), 73.5 (C_{5'}), 110.9, 116.0, 119.1, 137.8, 148.9, 153.7, 156.7 (adenine, C_{2'}, C_{1'}). ESI-MS 248 (M + H, 100.0), 270 (M + Na, 85.1). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

(-)-9-((Z)-(R)-2-[(R)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (ent-12a) and (+)-9-((E)-(R)-2-[(R)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (ent-13a). The reaction was performed as described above using the *Z,E*-isomers *ent-28a* (300 mg, 0.91 mmol) and K₂CO₃ (250 mg, 1.81 mmol) in methanol (18 mL) to give the *Z*-isomer *ent-12a* (94 mg, 42%) and *E*-isomer *ent-13a* (80 mg, 36%).

Z-Isomer *ent-12a*: mp 273–274 °C, $[\alpha]_D^{25} -56.6^\circ$ (*c* 1.13, DMSO). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

E-Isomer *ent-13a*: mp 251–252 °C, $[\alpha]_D^{26} 5.0^\circ$ (*c* 0.71, DMSO). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

(+)-9-((Z)-(S)-2-[(R)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (14a) and (-)-9-((E)-(S)-2-[(R)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (15a). The reaction was performed as in the previous experiments using the *Z,E*-isomers **29a** (260 mg, 0.7 mmol) to give the *Z*-isomer **14a** (68 mg, 35%) and *E*-isomer **15a** (93 mg, 48%).

Z-Isomer **14a**: mp 217–219 °C, $[\alpha]_D^{27} 94.7^\circ$ (*c* 0.46, DMSO). UV $\lambda_{\max}$ 278 nm (ϵ 8700), 261 (ϵ 12 800), 224 (ϵ 26 900). ¹H NMR δ 1.35–1.43 (m, 2H, H_{3'}), 2.10–2.15 (m, 1H, H_{4'}), 3.22–3.32 (m, 2H, H_{5'}, H_{6'}), 3.53–3.58 (m, 1H, H_{5'}), 4.66 (t, 1H, *J* = 5.6 Hz, 6'-OH), 4.91 (d, 1H, *J* = 4.8 Hz, 5'-OH), 7.32 (bs, 2H, NH₂), 7.36 (d, 1H, *J* = 1.6 Hz, H_{1'}), 8.16 (s, 1H, H₂), 8.46 (s, 1H, H₈). ¹³C NMR 5.8 (C_{3'}), 21.2 (C_{4'}), 66.0 (C_{6'}), 71.3 (C_{5'}), 110.3, 116.5, 119.0, 138.6, 148.9, 153.6, 156.7 (adenine, C_{2'}, C_{1'}). ESI-MS 248 (M + H, 100.0), 270 (M + Na, 111.2). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

E-isomer **15a**: mp 233–235 °C, $[\alpha]_D^{27} -5.8^\circ$ (*c* 0.43, DMSO). UV $\lambda_{\max}$ 278 nm (ϵ 8600), 262 (ϵ 11 700), 227 (ϵ 26 400). ¹H NMR δ 1.43–1.47 (m), 1.68 (dt, 2H, *J* = 8.8, 2.4 Hz, H_{3'}), 1.78–1.84 (m, 1H, H_{4'}), 3.04–3.09 (m, 1H), 3.42–3.45 (m, 2H, H_{5'}, H_{6'}), 4.69 (t, 1H, *J* = 4.8 Hz, 6'-OH), 4.90 (d, 1H, *J* = 4.8 Hz, 5'-OH), 7.33 (s, 2H, NH₂), 7.42 (s, 1H, H_{1'}), 8.15 (s, 1H, H₂), 8.47 (s, 1H, H₈). ¹³C NMR 9.6 (C_{3'}), 19.8 (C_{4'}), 66.4 (C_{6'}), 74.2 (C_{5'}), 111.3, 116.0,

119.1, 137.9, 148.9, 153.6, 156.7 (adenine, C_{2'}, C_{1'}). ESI-MS 248 (M + H, 100.0), 270 (M + Na, 111.9). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

(-)-9-((Z)-(S)-2-[(R)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (ent-14a) and (+)-9-((E)-(S)-2-[(R)-1,2-Dihydroxyethyl]cyclopropylidene)methyladenine (ent-15a). The experiment using the *Z,E*-isomers *ent-29a* (255 mg, 0.77 mmol) was performed as described above to give the *Z*-isomer *ent-14a* (97 mg, 51%) and *E*-isomer *ent-15a* (65 mg, 35%).

Z-Isomer *ent-14a*: mp 221–223 °C, $[\alpha]_D^{26} -97.2^\circ$ (*c* 0.48, DMSO). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

E-Isomer *ent-15a*: mp 230–231 °C, $[\alpha]_D^{28} 7.2^\circ$ (*c* 0.53, DMSO). Anal. (C₁₁H₁₃N₅O₂) C, H, N.

(+)-2-Amino-6-chloro-9-((Z)-(S)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene)methyl-purine (12c) and (+)-2-Amino-6-chloro-9-((E)-(S)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene)methyl-purine (13c). The *Z,E*-isomers **28b** (280 mg, 0.77 mmol) were dissolved in methanol (50 mL), NH₃ in MeOH (20%, 35 mL) was added, and the mixture was stirred for 13 h at room temperature. The volatiles were evaporated and the residue was chromatographed on a silica gel column in AcOEt–MeOH (60:1 to 20:1) to give the *Z*-isomer **12c** (90 mg, 41%) followed by *E*-isomer **13c** (105 mg, 48%).

Z-Isomer **12c**: mp 215–217 °C, $[\alpha]_D^{26} 80.9^\circ$ (*c* 0.75, DMF). UV $\lambda_{\max}$ 311 nm (ϵ 8100), 235 (ϵ 32 800). ¹H NMR δ 1.27 (poorly resolved m, 1H, H_{4'}), 3.11 (poorly resolved m, 1H), 3.80–3.47 (m, 2H, H_{5'}, H_{6'}), 4.70 (t, 1H, *J* = 4.8 Hz, 6'-OH), 5.14 (d, 1H, *J* = 4.8 Hz, 5'-OH), 7.01 (s, 2H, NH₂), 7.19 (s, 1H, H_{1'}), 8.91 (s, 1H, H₈). ¹³C NMR 6.8 (C_{3'}), 20.7 (C_{4'}), 66.4 (C_{6'}), 74.9 (C_{5'}), 110.3, 116.4, 123.7, 140.9, 150.2, 153.0, 160.7 (purine, C_{2'}, C_{1'}). ESI-MS 282, 284 (M + H, 100.0, 31.8), 304, 306 (M + Na, 82.7, 23.8).

E-Isomer **13c**: mp 258–260 °C, $[\alpha]_D^{25} 23.5^\circ$ (*c* 0.81, DMF). UV $\lambda_{\max}$ 311 nm (ϵ 8100), 235 (ϵ 32 800). ¹H NMR δ 1.42–1.45 (m), 1.67 (t, 2H, *J* = 8.8 Hz, H_{3'}), 1.83–1.88 (m, 1H, H_{4'}), 3.19 (poorly resolved m, 1H), 3.43 (poorly resolved m, 2H, H_{5'}, H_{6'}), 4.64 (t, 1H, *J* = 6.0 Hz, 6'-OH), 4.81 (d, *J* = 4.0 Hz, 5'-OH), 7.02 (s, 2H, NH₂), 7.33 (s, 1H, H_{1'}), 8.43 (s, 1H, H₈). ¹³C NMR 8.8 (C_{3'}), 19.0 (C_{4'}), 66.3 (C_{6'}), 73.3 (C_{5'}), 110.5, 117.0, 123.7, 140.2, 150.3, 153.1, 160.7 (purine, C_{2'}, C_{1'}). ESI-MS 282, 284 (M + H, 86.5, 25.2), 304, 306 (M + Na, 100.0, 31.4).

(-)-2-Amino-6-chloro-9-((Z)-(R)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene)methyl-purine (ent-12c) and (-)-2-Amino-6-chloro-9-((E)-(R)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene)methyl-purine (ent-13c). The above procedure starting from the *Z,E*-isomers *ent-28b* (340 mg, 0.93 mmol) afforded after chromatography in AcOEt–MeOH (30:1 to 20:1) the *Z*-isomer *ent-12c* (95 mg, 36%) followed by *E*-isomer *ent-13c* (130 mg, 50%).

Z-Isomer *ent-12c*: mp 210–212 °C, $[\alpha]_D^{28} -87.4^\circ$ (*c* 0.85, DMF).

E-Isomer *ent-13c*: mp 250–251 °C, $[\alpha]_D^{28} -25.3^\circ$ (*c* 0.67, DMF).

(+)-2-Amino-6-chloro-9-((Z)-(S)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene)methyl-purine (14c) and (+)-2-Amino-6-chloro-9-((E)-(S)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene)methyl-purine (15c). The procedure described above was performed with the *Z,E*-isomers **29b** (320 mg, 0.87 mmol) to give the faster moving *Z*-isomer **14c** (85 mg, 34%) and slower moving *E*-isomer **15c** (110 mg, 45%).

Z-Isomer **14c**: mp 205–207 °C, $[\alpha]_D^{24} 41.3^\circ$ (*c* 0.8, DMF). UV $\lambda_{\max}$ 311 nm (ϵ 7900), 234 (ϵ 31 700). ¹H NMR δ 1.34–1.43 (m, 2H, H_{3'}), 2.10–2.15 (m, 1H, H_{4'}), 3.20–3.31 (m, 2H), 3.47–3.50 (m, overlapped with H₂O, H_{5'}, H_{6'}), 4.63 (t, *J* = 5.2 Hz, 6'-OH), 4.86 (d, 1H, *J* = 4.0 Hz, 5'-OH), 7.00 (s, 2H, NH₂), 7.19 (d, 1H, *J* = 1.6 Hz, H_{1'}), 8.41 (s, 1H, H₈). ¹³C NMR 6.0 (C_{3'}), 21.3 (C_{4'}), 66.0 (H_{6'}), 71.4 (H_{5'}), 110.9, 117.5, 123.7, 140.9, 150.2, 153.3, 160.7 (purine, C_{2'}, C_{1'}). ESI-MS (MeOH + AcOK) 282, 284 (M + H, 16.6, 5.0), 320, 322 (M + K, 100.0, 41.4).

E-Isomer **15c**: mp 243–245 °C, $[\alpha]_D^{25} = 51.8^\circ$ (*c* 1.1, DMF). UV $\lambda_{\max}$ 311 nm (ϵ 8000), 234 (ϵ 32 200). ¹H NMR δ 1.42–1.45 (m), 1.69 (t, *J* = 8.8 Hz, 2H, H_{3'}), 1.77–1.82 (m, 1H, H_{4'}), 3.03 (m, 1H), 3.50 (m, partly overlapped with H₂O, 2H, H_{5'}, H_{6'}), 4.67 (poorly resolved t, 1H, 6'-OH), 4.89 (d, *J* = 4.8 Hz, 5'-OH), 7.02

(s, 2H, NH₂), 7.25 (s, 1H, H_{1'}), 8.43 (s, 1H, H₈). ¹³C NMR 9.7 (C_{3'}), 20.0 (C_{4'}), 66.4 (C_{6'}), 74.2 (C_{5'}), 111.0, 116.9, 123.7, 140.2, 150.3, 153.1, 160.7 (purine, C_{2'}, C_{1'}). ESI-MS 304, 306 (M + Na⁺, 100.0, 32.5), 585, 587 (2 M + Na, 57.4, 39.9).

(-)-**2-Amino-6-chloro-9-[(Z)-(R)-2-[(S)-1,2-dihydroxyethyl]-cyclopropylidene]methyl]purine (ent-14c)** and (-)-**2-Amino-6-chloro-9-[(E)-(R)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene]methyl]purine (ent-15c)**. The experiment described above was performed with the *Z,E*-enantiomers *ent-29b* (300 mg, 0.82 mmol) to furnish the *Z*-isomer *ent-14c* (94 mg, 41%) and *E*-isomer *ent-15c* (106 mg, 46%).

Z-Isomer *ent-14c*: mp 199–200 °C, [α]_D²⁷ -39.3° (c 0.75, DMF).

E-Isomer *ent-15c*: mp 248–250 °C, [α]_D²⁷ -49.7° (c 1.0, DMF).

(+)-**9-[(Z)-(S)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (12b)**. A solution of the *Z*-isomer *ent-12c* (85 mg, 0.3 mmol) in 80% formic acid (80%, 8 mL) was heated at 80 °C for 4 h. The volatiles were evaporated in vacuo, the crude product was dissolved in NH₃ in methanol (5%, 40 mL), and the mixture was stirred for 30 min at 0 °C. After removal of solvents, the solid was recrystallized from NH₄OH (28%) to give the guanine *Z*-isomer **12b** (66 mg, 83%): mp >300 °C, [α]_D²⁷ 98.6° (c 0.56, DMSO). UV λ_{max} 271 nm (ε 10 000), 231 (ε 28 100). ¹H NMR δ 1.22 (m, 1.42 (t, 2H, *J* = 8.0 Hz, H_{3'}), 1.94 (m, 1H, H_{4'}), 3.10 (m, 1H), 3.39–3.46 (m, 2H, partly overlapped with H₂O, H_{5'}, H_{6'}), 4.68 (bs, 1H, 6'-OH), 5.10 (bs, 1H, 5'-OH), 6.51 (s, 2H, NH₂), 7.10 (s, 1H, H_{1'}), 8.56 (s, 1H, H₈), 10.64 (bs, 1H, NH). ¹³C NMR 6.6 (C_{3'}), 20.7 (C_{4'}), 66.5 (C_{6'}), 75.0 (C_{5'}), 110.8, 115.1, 116.8, 135.4, 150.3, 154.5, 157.4 (guanine, C_{2'}, C_{1'}). ESI-MS 264 (M + H, 100.0), 286 (M + Na, 65.7). Anal. (C₁₁H₁₃N₅O₃·0.5H₂O) C, H, N.

(-)-**9-[(Z)-(R)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (ent-12b)**. The procedure described above was performed with the *Z*-isomer *ent-12c* (90 mg, 0.32 mol) to give the title compound *ent-12b* (73 mg, 87%): mp >300 °C, [α]_D²⁷ -101.7° (c 0.52, DMSO). Anal. (C₁₁H₁₃N₅O₃·0.45H₂O) C, H, N.

(+)-**9-[(E)-(S)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (13b)**. The procedure described above was performed with the *E*-isomer *ent-13c* (90 mg, 0.32 mmol) to give the title compound **13b** (71 mg, 84%): mp >300 °C, [α]_D²⁷ 8.8° (c 0.8, DMSO). UV λ_{max} 271 nm (ε 10 900), 231 (ε 28 200). ¹H NMR δ 1.36–1.40 (m), 1.61 (dt, 2H, *J* = 8.8, 1.6 Hz, H_{3'}), 1.79–1.84 (m, 1H, H_{4'}), 3.17 (m, 1H), 3.40–3.45 (m, partly overlapped with H₂O, 2H, H_{5'}, H_{6'}), 4.62 (t, 1H, *J* = 4.8 Hz, 6'-OH), 4.78 (d, 1H, *J* = 4.8 Hz, 5'-OH), 6.53 (s, 2H, NH₂), 7.24 (s, 1H, H_{1'}), 8.02 (s, 1H, H₈), 10.67 (bs, 1H, NH). ¹³C NMR 8.5 (C_{3'}), 18.8 (C_{4'}), 66.3 (C_{6'}), 73.4 (C_{5'}), 110.8, 115.5, 116.9, 134.4, 150.5, 154.6, 157.4 (guanine, C_{2'}, C_{1'}). ESI-MS 264 (M + H, 81.4), 286 (M + Na, 100.0). Anal. (C₁₁H₁₃N₅O₃·0.5H₂O).

(-)-**9-[(E)-(R)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (ent-13b)**. The procedure described above was performed with the *E*-isomer *ent-13c* (110 mg, 0.39 mol) to give the title compound *ent-13b* (86 mg, 83%): mp >300 °C, [α]_D²⁷ -10.8° (c 0.88, DMSO). Anal. (C₁₁H₁₃N₅O₃·0.45H₂O) C, H, N.

(+)-**9-[(Z)-(S)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (14b)**. The above protocol was performed with the *Z*-isomer *ent-14c* (80 mg, 0.28 mmol) to give the title compound **14b** (64 mg, 86%), mp >300 °C, [α]_D²⁷ 62.4° (c 0.53, DMSO). UV λ_{max} 271 nm (ε 9700), 230 (ε 22 800). ¹H NMR δ 1.30–1.38 (m, 2H, H_{3'}), 2.05–2.09 (m, 1H, H_{4'}), 3.25–3.31 (m, 2H, partially overlapped with H₂O), 3.51 (m, 1H, H_{5'}, H_{6'}), 4.64 (s, 1H, 6'-OH), 4.85 (s, 1H, 5'-OH), 6.53 (s, 2H, NH₂), 7.09 (d, 1H, *J* = 1.6 Hz, H_{1'}), 8.01 (s, 1H, H₈), 10.71 (bs, 1H, NH). ¹³C NMR 5.7 (C_{3'}), 20.9 (C_{4'}), 66.0 (C_{6'}), 71.4 (C_{5'}), 111.2, 116.0, 116.9, 135.1, 150.6, 154.6, 157.5 (guanine, C_{2'}, C_{1'}). ESI-MS 264 (M + H, 100.0), 286 (M + Na, 39.6). Anal. (C₁₁H₁₃N₅O₃·0.5H₂O) C, H, N.

(-)-**9-[(Z)-(R)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (ent-14b)**. The procedure described above was performed with the *E*-isomer *ent-13c* (85 mg, 0.3 mol) to give the title compound *ent-14b* (68 mg, 86%): mp >300 °C, [α]_D²⁷ -62.3° (c 0.55, DMSO). Anal. (C₁₁H₁₃N₅O₃·0.15H₂O) C, H, N.

(+)-**9-[(E)-(S)-2-[(R)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (15b)**. The procedure described above was performed with the *E*-isomer *ent-15c* (100 mg, 0.35 mmol) to give the title compound **15b** (78 mg, 83%): mp >300 °C, [α]_D²⁷ 17.5° (c 0.31, DMSO). UV λ_{max} 270 nm (ε 9400), 231 (ε 24 300). ¹H NMR δ 1.35–1.40 (m, 1H), 1.64 (poorly resolved dt, 1H, H_{3'}), 1.72–1.77 (m, 1H, H_{4'}), 2.97–3.03 (m, 1H), 3.37–3.46 (m, partly overlapped with H₂O, 2H, H_{5'}, H_{6'}), 4.63 (t, 1H, *J* = 5.2 Hz, 6'-OH), 4.85 (d, 1H, *J* = 4.0 Hz, 5'-OH), 6.54 (s, 2H, NH₂), 7.17 (s, 1H, H_{1'}), 8.04 (s, 1H, H₈), 10.64 (s, 1H, NH). ¹³C NMR 9.5 (C_{3'}), 19.8 (C_{4'}), 66.4 (C_{6'}), 74.3 (C_{5'}), 111.3, 115.5, 116.9, 134.4, 150.5, 154.6, 157.4 (guanine, C_{2'}, C_{1'}). ESI-MS 264 (M + H, 47.0), 286 (M + Na, 100.0). Anal. (C₁₁H₁₃N₅O₃·0.5H₂O) C, H, N.

(-)-**9-[(E)-(R)-2-[(S)-1,2-dihydroxyethyl]cyclopropylidene]methyl]guanine (ent-15b)**. The procedure described above was performed with the *E*-isomer *ent-15c* (95 mg, 0.34 mol) to give the title compound *ent-15b* (75 mg, 84%): mp >300 °C, [α]_D²⁷ -19.4° (c 0.41, DMSO). Anal. (C₁₁H₁₃N₅O₃·0.4H₂O) C, H, N.

Benzyl (1*S*,2*R*,5*S*)-5-Bromo-3-oxabicyclo[3.1.0]hexane-2-carboxylate (30). Pyridinium tribromide (85 mg, 0.27 mmol) was added with stirring to a solution of (1*S*,2*S*)-ester **20** (50 mg, 0.23 mmol) in CH₂Cl₂ (1 mL) at 0 °C. The stirring was continued for 1 h at room temperature. The mixture was then filtered through a silica gel pad (1 cm), which was washed with CH₂Cl₂ (10 mL). The solvent was evaporated, and the crude dibromide **32** and K₂CO₃ (50 mg, 0.36 mmol) were refluxed in THF (1.5 mL) with stirring for 5 min. After cooling, the mixture was chromatographed on a silica gel column in hexanes–Et₂O (10:1) to furnish compound **30** (22 mg, 32%) as a colorless oil. [α]_D²⁵ -17.1° (c 0.70, CHCl₃). ¹H NMR (CDCl₃) δ 1.14 (t, *J* = 5.6 Hz), 1.38 (dd, 2H, *J* = 8.0 Hz, H₃), 2.10 (dd, 1H, *J* = 9.0, 5.0 Hz, H₂), 4.23, 4.10 (AB, 2H, *J*_{AB} = 7.8 Hz, H₅), 4.31 (s, 1H, H₁), 5.26, 5.20 (AB, 2H, *J*_{AB} = 12.0 Hz, CH₂Ph), 7.37 (m, 5H, Ph). ¹³C NMR 18.4 (C₆), 29.1 (C₁), 30.1 (C₅), 67.2 (C₄), 75.0 (CH₂Ph), 78.1 (C₂), 128.4, 128.7, 128.9, 135.5 (Ph), 171.5 (C=O). ESI-MS 319, 321 (M + Na, 100.0, 100.0), 615, 617, 619 (2M + Na, 38.0, 76.7, 40.0). Anal. (C₁₃H₁₃BrO₃) C, H.

Benzyl (1*R*,2*R*,5*S*)-5-Bromo-3-oxabicyclo[3.1.0]hexane-2-carboxylate (31). The above-described protocol was followed with (1*S*,2*R*)-ester **21** (85 mg, 0.39 mmol) via dibromide **33** to give compound **31** (40 mg, 35%) as a colorless oil: [α]_D²⁵ 48.5° (c 0.86, CHCl₃). ¹H NMR (CDCl₃) δ 1.23 (t, 1H, *J* = 7.6 Hz), 1.34 (t, 1H, *J* = 5.8 Hz, H₆, H_{6'}), 2.19 (ddd, 1H, *J* = 8.0, 4.4, 1.6 Hz, H₁), 4.24, 3.98 (AB, 2H, *J*_{AB} = 8.6 Hz, H₄), 4.67 (d, 1H, *J* = 3.2 Hz, H₂), 5.20 (d, 2H, *J* = 1.6 Hz, CH₂Ph), 7.35 (m, 5H, Ph). ¹³C NMR 15.5 (C₆), 28.6 (C₁), 30.7 (C₅), 67.2 (C₄), 75.6 (CH₂Ph), 76.9 (C₂), 128.5, 128.7, 128.9, 135.5 (Ph), 169.4 (C=O). ESI-MS 319, 321 (M + Na, 100.0, 97.4), 615, 617, 619 (2M + Na, 23.7, 48.7, 25.7). Anal. (C₁₃H₁₃BrO₃) C, H.

(Z)-9-[(R)-2-[(R)-1,2-diacetoxyethyl]cyclopropylidene]methyl]adenine (34). A mixture of the *Z*-isomer *ent-12a* (16 mg, 0.065 mmol) and Ac₂O (0.3 mL) in pyridine (1 mL) was stirred for 5 h at room temperature. Solvent was removed at room temperature and 0.04 torr. The crude product was chromatographed on a silica gel column in AcOEt–methanol (50:1 to 20:1) to give compound **34** (16 mg, 75%). Single crystals for X-ray diffraction were obtained by slow crystallization of a solution in AcOEt: mp 185–186 °C, [α]_D²⁵ -152.6° (c 1.03, MeOH). UV λ_{max} 278 nm (ε 8600), 261 (ε 12 500), 227 (ε 28 200). ¹H NMR (CDCl₃) δ 1.43–1.46 (m), 1.70 (dt, 2H, *J* = 8.8, 1.6 Hz, H_{3'}), 1.92, 2.06 (2s, 6H, CH₃), 2.25–2.31 (m, 1H, H_{4'}), 4.43, 4.17 and 4.42, 4.18 (2AB, 2H, *J*_{AB} = 11.6 Hz, H_{6'}), 4.67 (m, 1H, H_{5'}), 5.97 (bs, 2H, NH₂), 7.49 (d, 1H, *J* = 1.6 Hz, H_{1'}), 8.38 (s, 2H, H₂, H₈). ¹³C NMR 7.6 (C_{3'}), 17.6 (C_{4'}), 21.0 (CH₃), 64.6 (C_{6'}), 74.5 (C_{5'}), 112.1, 113.1, 119.5, 137.7, 149.1, 153.8, 155.8 (adenine, C_{1'}, C_{2'}), 170.0, 170.8 (C=O). ESI-MS: 332 (M + H, 68.9), 354 (M + Na, 100.0), 53.0, 685 (2M + Na, 55). Anal. (C₁₅H₁₇N₅O₄) C, H, N.

Antiviral Assays. Antiviral assays were performed as described previously.^{29,30} HCMV assays were run in human foreskin fibroblast (HFF) cell culture with two strains of virus, Towne and AD169, in a plaque reduction or cytopathic effect (CPE)

inhibition assay. Virus strains resistant to ganciclovir (**10**) and cyclopropavir (**3b**) were isolated from Towne strain by growth in synadenol (**1a**) (strain E8)²³ or in **3b** (isolate 2696r).²⁴ The former strain has two point mutations introduced into gene UL97; the latter has a truncated UL97 gene. The MCMV was assayed in mouse embryonic fibroblast (MEF) cells by plaque reduction. The EBV DNA hybridization assay was run in Akata cells.^{30,31}

Adenosine Deaminase (ADA) Assay.²⁹ Adenine analogues **12a–15a** and *ent*-**12a** to *ent*-**15a** (2 μ mol) and ADA from calf intestine (1.7 units) were magnetically stirred in 0.05 M Na₂HPO₄ (pH 7.5) in a total volume of 0.6 mL. Aliquots were removed and diluted with buffer. UV spectra were recorded, and a decrease of absorbance at 260 nm was followed. The results are summarized in Table 7.

Acknowledgment. We thank M. J. Heeg from the Department of Chemistry, Wayne State University, for X-ray crystallographic studies and L. M. Hryhorczuk from the Central Instrumentation Facility, Department of Chemistry, Wayne State University, for mass spectra. We also thank Kathy Borysko and Julie Breitenbach of the University of Michigan for isolation and engineering of drug-resistant HCMV and for the antiviral assays. The work described herein was supported by U.S. Public Health Service Grant RO1-CA32779 and Contract NO1-AI-30049 from the National Institutes of Health, Bethesda, Maryland.

Supporting Information Available: Crystallographic data of diacetate **34** (Tables 1–6) and elemental analysis results of all biologically tested compounds and some key intermediates (Table 7). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Zemlicka, J. Methylenecyclopropane Analogues of Nucleosides as Anti-Herpes Agents. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; Elsevier: Amsterdam, The Netherlands, 2007; pp 113–165.
- (2) Zhou, S.; Breitenbach, J. M.; Borysko, K. Z.; Drach, J. C.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Zemlicka, J. Synthesis and Antiviral Activity of (Z)- and (E)-2,2-[Bis(hydroxymethyl)cyclopropylidene]methylpurines and -pyrimidines: Second-Generation Methylenecyclopropane Analogues of Nucleosides. *J. Med. Chem.* **2004**, *47*, 566–575.
- (3) Kern, E. R.; Kushner, N. L.; Hartline, C. B.; Williams-Azziz, S. L.; Harden, E. A.; Zhou, S.; Zemlicka, J.; Prichard, M. N. In Vitro Activity and Mechanism of Action of Methylenecyclopropane Analogs of Nucleosides against Herpesvirus Replication. *Antimicrob. Agents Chemother.* **2005**, *49*, 1039–1045.
- (4) Kern, E. R.; Bidanset, D. J.; Hartline, C. B.; Yan, Z.; Zemlicka, J.; Quenelle, D. C. Oral Activity of a Methylenecyclopropane Analog, Cyclopropavir, in Animal Models for Cytomegalovirus Infections. *Antimicrob. Agents Chemother.* **2004**, *48*, 4745–4753.
- (5) Bowlin, T.; Brooks, J.; Zemlicka, J. Preclinical Pharmacokinetic, Toxicokinetic and Toxicology Results for Cyclopropavir, a Promising New Agent for the Treatment of Beta- and Gamma-Herpesviruses. Presented at the 22nd International Conference on Antiviral Research, Miami, FL, May 3–7, 2009; Abstract 99. Bowlin, T.; Brooks, J.; Zemlicka, J. *Antiviral Res.*, in press.
- (6) Chen, X.; Matsumi, S.; Mitsuya, H.; Zemlicka, J. Synthesis of (Z)- (2,3-bis-Hydroxymethyl)methylenecyclopropane Analogues of Purine Nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 265–274.
- (7) Cheng, C.; Shimo, T.; Somekawa, K.; Kawaminami, M. Reactions of Methylenecyclopropanes with Dialkylzinc-Bromoform System, and the Utilization for Synthesis of a Novel Cyclopropylidene-Nucleoside. *Tetrahedron Lett.* **1997**, *38*, 9005–9008.
- (8) Zhou, S.; Zemlicka, J. Synthesis of 2,2,3-Tris(hydroxymethyl)methylenecyclopropane Analogues of Nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 391–402.
- (9) Ashton, W. T.; Canning, L. F.; Reynolds, G. F.; Tolman, R. L.; Karkas, J. D.; Liou, R.; Davies, M.-L. M.; DeWitt, C. M.; Perry, H. C.; Field, A. K. Synthesis and Antitherapeutic Activity of (S)-, (R)-, and ($\pm$)-9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine, Linear Isomers of 2'-Nor-2'-deoxyguanosine. *J. Med. Chem.* **1985**, *28*, 926–933.
- (10) Karkas, J. D.; Ashton, W. T.; Canning, L. F.; Liou, R.; Germerhausen, J.; Bostedor, R.; Arison, B.; Field, A. K.; Tolman, R. L. Enzymatic Phosphorylation of the Antitherapeutic Agent 9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine. *J. Med. Chem.* **1986**, *29*, 842–848.
- (11) Okuma, K.; Tanaka, Y.; Yoshihara, K.; Ezaki, A.; Koda, G.; Ohta, H.; Hara, K.; Kashimura, S. *J. Org. Chem.* **1993**, *58*, 5037–5039.
- (12) Le Corre, M.; Hercouet, A.; Bessieres, B. New Convenient Access to Optically Active Methylenecyclopropylcarbinols. *J. Org. Chem.* **1994**, *59*, 5483–5484.
- (13) Chen, X.; Zemlicka, J. Revision of Absolute Configuration of Enantiomeric (Methylenecyclopropyl)carbinols Obtained from (R)-(-) and (S)-(+)-Epichlorohydrin and Methylenetriphenylphosphorane. Implications for Reaction Mechanism and Improved Synthesis of Methylenecyclopropane Analogues of Nucleosides. *J. Org. Chem.* **2002**, *67*, 286–289.
- (14) de Meijere, A.; Bagutski, V.; Zeuner, F.; Fischer, U. K.; Rheinberger, V.; Moszner, N. Synthesis and Polymerization of Various 2-Cyclopropylacrylates. *Eur. J. Org. Chem.* **2004**, 3669–3678.
- (15) Wang, B.-Y.; Huang, J.-W.; Liu, L.-P.; Shi, M. Highly Efficient and Stereoselective Construction of 1-Iodo- or 1-Phenylselenenyl-2-aryl-3-oxabicyclo[3.1.0]hexane from the Reaction of Arylmethylenecyclopropylcarbinols with Iodine or Diphenyldiselenide. *Synlett* **2005**, 421–424.
- (16) Zemlicka, J.; Chen, X. Methylenecyclopropane Analogues of Nucleosides as Antiviral Agents. In *Frontiers in Nucleosides and Nucleic Acids*; Schinazi, R. F.; Liotta, D. C., Eds.; IHL Press: Tucker, GA, 2004; pp 267–307.
- (17) Guan, H.-P.; Qiu, Y.-L.; Ksebati, M. B.; Kern, E. R.; Zemlicka, J. Synthesis of phosphonate derivatives of methylenecyclopropane nucleoside analogues by alkylation–elimination method and unusual opening of cyclopropane ring. *Tetrahedron* **2002**, *58*, 6047–6059.
- (18) Zhou, S. M.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Drach, J. C.; Tamiya, S.; Mitsuya, H.; Zemlicka, J. 9-[[3-Fluoro-2-(hydroxymethyl)cyclopropylidene]methyl]-adenines and -guanines. Synthesis and Antiviral Activity of All Stereoisomers. *J. Med. Chem.* **2006**, *49*, 6120–6128.
- (19) Norbeck, D. W.; Rosen, T. J.; Sham, H. L. Preparation of Cyclopropyl Nucleoside Analogs with Antiviral Activity. U.S. Patent 4,988,703, 1989. *Chem. Abstr.* **1991**, *115*, 50218.
- (20) Zhao, Y.; Yang, T.; Lee, M.; Lee, D.; Newton, M. G.; Chu, C. K. Asymmetric Synthesis of (1'S,2'R)-Cyclopropyl Carbocyclic Nucleosides. *J. Org. Chem.* **1995**, *60*, 5236–5242.
- (21) Pierra, C.; Olgen, S.; Cavalcanti, S. C. H.; Cheng, Y.-C.; Schinazi, R. F.; Chu, C. K. Synthesis and Antiviral Activities of Enantiomeric 1-(2-Hydroxymethyl)cyclopropylmethyl Nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 253–268.
- (22) Baldanti, F.; Sarasini, A.; Drach, J. C.; Zemlicka, J.; Gerna, G. Z-Isomers of 2-hydroxymethylcyclopropylidenemethyl adenine (synadenol) and guanine (synguanol) are active against ganciclovir- and foscarnet-resistant human cytomegalovirus UL97 mutants. *Antiviral Res.* **2002**, *56*, 273–278.
- (23) Breitenbach, J. M.; Borysko, K. Z.; Zemlicka, J.; Drach, J. C. Resistance of Human Cytomegalovirus with Single and Double Mutations in UL97 to First and Second Generation of Methylenecyclopropane Purines. Presented at the 19th International Conference on Antiviral Research, San Juan, Puerto Rico, May 7–11, 2006; Abstract 61. Breitenbach, J. M.; Borysko, K. Z.; Zemlicka, J.; Drach, J. C. *Antiviral Res.* **2006**, *70*, A69.
- (24) Borysko, K. Z.; Breitenbach, J. M.; Gentry, B. G.; Zemlicka, J.; Drach, J. C. Selection of Human Cytomegalovirus Resistant to a Second Generation Methylenecyclopropane Purine. Presented at the 20th International Conference on Antiviral Research, Palm Springs, CA, April 29 through May 3, 2007; Abstract 139. Borysko, K. Z.; Breitenbach, J. M.; Gentry, B. G.; Zemlicka, J.; Drach, J. C. *Antiviral Res.* **2007**, *74*, A83.
- (25) Borysko, K. Z.; Gentry, B. G.; Breitenbach, J. M.; Zemlicka, J.; Drach, J. C. Resistance of Human Cytomegalovirus to Cyclopropavir Involves a Novel Mutation in UL97. Presented at the 21st International Conference on Antiviral Research, Montreal, Quebec, Canada, April 13–17, 2008; Abstract 98. Borysko, K. Z.; Gentry, B. G.; Breitenbach, J. M.; Zemlicka, J.; Drach, J. C. *Antiviral Res.* **2008**, *78*, A54.
- (26) Sullivan, V.; Talarico, C. L.; Stanat, S. C.; Davis, M.; Coen, D. M.; Biron, K. K. A Protein Kinase Homologue Controls Phosphorylation of Ganciclovir in Human Cytomegalovirus-Infected Cells. *Nature (London)* **1992**, *358*, 162–164.
- (27) Furrow, M. E.; Schaus, S. E.; Jacobsen, E. N. Practical Access to Highly Enantioenriched C-3 Building Blocks via Hydrolytic Kinetic Resolution. *J. Org. Chem.* **1998**, *63*, 6776–6777.
- (28) Brugat, N.; Duran, J.; Polo, A.; Real, J.; Alvarez-Larena, A.; Piniella, J. F. Synthesis and Characterization of a New Chiral Phosphinothiol Ligand and Its Palladium(II) cComplexes. *Tetrahedron: Asymmetry* **2002**, *13*, 569–577.

- (29) Zhou, S.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Drach, J. C.; Matsumi, S.; Mitsuya, H.; Zemlicka, J. (Z)- and (E)-[2-Fluoro-2-(hydroxymethyl)cyclopropylidene]methyl-purines and -pyrimidines, a New Class of Methylenecyclopropane Analogues of Nucleosides: Synthesis and Antiviral Activity. *J. Med. Chem.* **2004**, *47*, 6964–6972.
- (30) Li, C.; Prichard, M. N.; Korba, B. E.; Drach, J. C.; Zemlicka, J. Fluorinated Methylenecyclopropane Analogues of Nucleosides. Synthesis and Antiviral Activity of (Z)- and (E)-9-[(2-Fluoromethyl-2-hydroxymethyl)cyclopropylidene]methyl}adenine and -guanine. *Bioorg. Med. Chem.* **2008**, *16*, 2148–2155.
- (31) Prichard, M. N.; Daily, S. L.; Jefferson, G. L.; Perry, A. L.; Kern, E. R. A Rapid DNA Hybridization Assay for the Evaluation of Antiviral Compounds against Epstein–Barr Virus. *J. Virol. Methods* **2007**, *144*, 86–90.

JM900126V