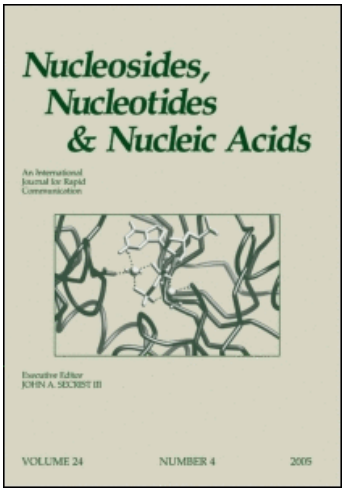


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis Of 2,2,3-Tris(Hydroxymethyl)Methylenecyclopropane Analogues Of Nucleosides

Shaoman Zhou[a]; Jiri Zemlicka[a]
[a] Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan, USA

To cite this Article Zhou, Shaoman and Zemlicka, Jiri(2007) 'Synthesis Of 2,2,3-Tris(Hydroxymethyl)Methylenecyclopropane Analogues Of Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 26: 4, 391 — 402
To link to this Article: DOI: 10.1080/15257770701297000
URL: http://dx.doi.org/10.1080/15257770701297000

Nucleosides, Nucleotides, and Nucleic Acids, 26:391–402, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701297000

Taylor & Francis
Taylor & Francis Group

SYNTHESIS OF 2,2,3-TRIS(HYDROXYMETHYL)METHYLENECYCLOPROPANE ANALOGUES OF NUCLEOSIDES

Shaoman Zhou and Jiri Zemlicka □ *Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan, USA*

□ *Synthesis of 2,2,3-tris(hydroxymethyl)methylenecyclopropane analogues* ***16a***, ***16b***, ***17a***, *and* ***17b*** *is described. Diethyl ester of Feist's acid* ***18b*** *was hydroxymethylated via carbanion formation using formaldehyde under simultaneous isomerization to cis diester to give intermediate* ***19***. *Reduction followed by acetylation gave triacetate* ***22***. *Addition of bromine afforded reagent* ***23***, *which was used for alkylation-elimination of adenine and 2-amino-6-chloropurine to provide Z,E-isomeric mixtures of* ***24a*** *and* ***24b***. *Deacetylation and separation furnished the Z-isomers* ***16a***, ***16c*** *and E-isomers* ***17a***, ***17c***. *Hydrolytic dechlorination of* ***16c*** *and* ***17c*** *gave guanine analogues* ***16b*** *and* ***17b***. *None of the analogues exhibited a significant antiviral activity. Adenosine deaminase is refractory toward adenine analogues* ***16a*** *and* ***17a***.

Keywords Tris(hydroxymethyl)methylenecyclopropanes; alkylation-elimination; nucleoside analogues; antivirals

INTRODUCTION

In recent years, we have been delineating the structure-activity relationships (SAR) of a new class of antiviral nucleoside analogues where the tetrahydrofuran ring of a ribofuranose or 2-deoxyribofuranose moiety was replaced with a methylenecyclopropane unit.[1,2] The purine *Z*-isomers of the first generation analogues **1** are particularly effective against human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), human herpes virus 6 (HHV-6), and human herpes virus 8 (HHV-8, Chart 1). The *E*-isomers **2** are active only exceptionally. The *Z*-isomers of the second generation

Received 19 October 2006; accepted 24 January 2007.

We thank L. M. Hrihorczuk from the Central Instrumentation Facility, Department of Chemistry, Wayne State University (D. M. Coleman, Director) for mass spectra. The work described herein was supported by U.S. Public Health Service grants RO1-CA32779 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

Address correspondence to Jiri Zemlicka, Barbara Ann Karmanos Cancer Institute, 110 E. Warren Ave., Detroit, MI48201-1379. E-mail: zemlicka@karmanos.org

Chart 1

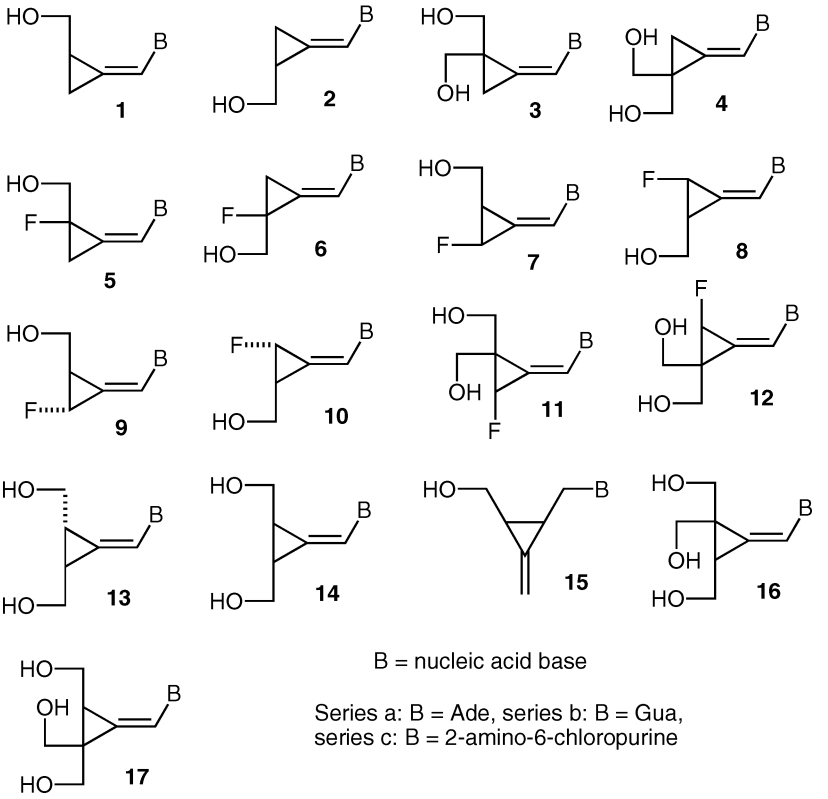


CHART 1

series **3** have a more narrow antiviral potency, but the guanine analogue, cyclopropavir **3b**, is the most effective anti-HCMV agent[3,4] from all the methylenecyclopropanes described to date. The *E*-isomers **4** (B = 2,6-diaminopurine and 2-amino-6-cyclopropylaminopurine) inhibit replication of EBV.

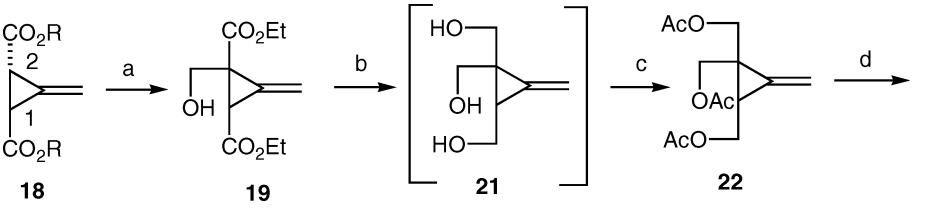
These results gave impetus for studies of SAR of methylenecyclopropane analogues with a particular emphasis on substituents in the cyclopropane moiety. Thus, in the series of fluorinated analogues the antiviral activity of the *Z*(*cis*) isomers follows roughly the order[5–7] **5** > **7** > **9** > **11**. The *E*(*trans*)-isomers **6a**, **6b**, **6c**, and **10a** are potent anti-EBV agents. Analogues **8**, **10**, and **12** are without significant effect. Previously, analogue **13a** also was synthesized but no biological data were reported.[8] The corresponding *cis*-isomer **14a** exhibited a moderate anti-HIV effect.[9] Methylenecyclopropanes **15** with a purine residue attached to a side chain are devoid of antiviral effcts.[10] In this communication, we describe the synthesis of adenine and guanine analogues **16a**, **17a** and **16b**, **17b**, which are related to compounds **3**, **4** or **13**, **14** having an additional hydroxymethyl function.

A major difference is an absence of *cis, trans* isomerism in the cyclopropane moiety of analogues **16** and **17**.

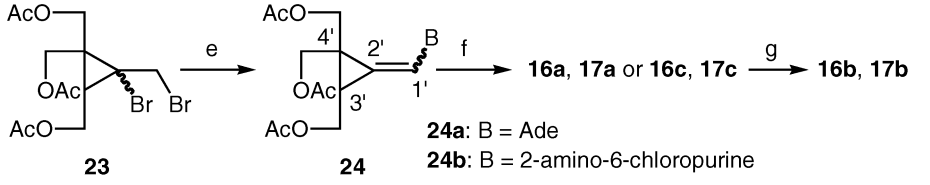
RESULTS AND DISCUSSION

Synthesis

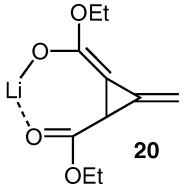
3-Methylenecyclopropane-*trans*-1,2-dicarboxylic acid (Feist's acid, **18a**)[11] served as a convenient starting material. It was converted to diethyl ester **18b** (Scheme 1) in 91% yield by the method briefly described for the dimethyl ester.[10] For the hydroxymethylation, the procedure previously used[5,12] for electrophilic fluorination of methylenecyclopropane carboxylates was adapted for hydroxymethylation. Diester **18b** was converted to the respective carbanion using LDA and excess LiCl in THF at −78°C which was then reacted with gaseous formaldehyde to give hydroxymethyl derivative **19** in 44% yield. Simultaneously, the



18a: R = H **18b**: R = Et



a. 1. LiCl, LDA, THF, -78 °C. 2. CH_2O (g).
b. $LiAlH_4$, Et_2O.
c. Ac_2O, pyridine.
d. Pyridine.HBr_3, CH_2Cl_2.
e. B-H, K_2CO_3, DMF, Δ.
f. K_2CO_3, MeOH (**24a**) or NH_3, MeOH, 0 °C (**24b**).
g. 1. 80% HCO_2H, Δ. 2. NH_3, MeOH, 0 °C.



SCHEME 1

trans configuration of the carbethoxy groups originally present in **18a** and **18b** was transformed to *cis*. This was evident from the ^{1}H NMR and NOE experiment with **19**. Thus, irradiation of methylene protons of CH_2OH at δ 3.65 and 3.79 led to NOE enhancement at δ 2.59 (H_1) of 3.58 and 3.63%, respectively. This indicated a *cis* relationship of CH_2OH and H_1. It is reasonable to assume that an intermediary *cis* lithium enolate **20** will be more stable than the *trans* isomer providing thus a driving force for the isomerization.[13] Nevertheless, this stereochemistry disappeared in the next step of the synthesis, reduction of **19**. The latter reaction gave crude triol **21** which was directly acetylated to give triacetoxy derivative **22** in 88% yield. The alkylation-elimination protocol followed the established pattern.[1,2] Addition of bromine via pyridinium perbromide in CH_2Cl_2 gave the reagent **23** (90%). Reaction with adenine using K_2CO_3 in DMF gave the expected mixture of the *Z*- and *E*-isomers **24a** (ratio 1.6 : 1, 56% yield) inseparable by column chromatography on silica gel. Deacetylation using K_2CO_3 in methanol gave the target *Z*- and *E*-isomers **16a** and **17a** in 47 and 39% yield, respectively, after chromatographic separation. In a similar fashion, alkylation-elimination of 2-amino-6-chloropurine with **23** gave triacetoxy intermediate **24b** (*Z/E* = 1.4 : 1, 67% yield). Deprotection furnished after separation compounds **16c** and **17c** (44 and 27%). Hydrolysis of **16c** and **17c** with 80% formic acid afforded guanine analogues **16b** and **17b** in 87and 83% yield, respectively.

NMR Spectra and *E, Z* Isomeric Assignment

As in the previous cases of methylenecyclopropane analogues[1,2] NMR spectra were indispensable for *E,Z* isomeric assignment. Nonequivalency of the CH_2OH functions was observed in the ^{1}H NMR of the bis-hydroxymethyl compounds **3** and **4** but the corresponding carbon atoms in the ^{13}C NMR were equivalent.[3] This was not the case of analogues **16** and **17** where all the methylene protons and carbons are nonequivalent. It is recognized that unlike **3** and **4** analogues **16** and **17** are chiral. Therefore, the 4′- CH_2OH groups of the latter compounds are diastereotopic. Indeed, three different CH_2OH functions were observed in the ^{13}C NMR spectra of analogues **16a**, **16b**, **16c** and **17a**, **17b**, **17c** irrespective of their *Z,E* isomerism. The ^{1}H NMR patterns of the CH_2OH groups were complex showing that virtually all methylene protons are non-equivalent. This was also seen in triacetate **22** where the ^{13}C NMR indicated the presence of three methylene groups whereas a total of six methylene groups were noted in triacetates **24a** and **24b** which were obtained only as *Z,E* isomeric mixtures.

Patterns of chromatographic mobility and chemical shifts of the relevant NMR signals provided valuable tools for preliminary assignment of the *Z*- and *E*-isomers of a number of methylenecyclopropane analogues of

TABLE 1 Chemical shifts (δ) of the relevant ^{1}H NMR signals of 2,2-disubstituted and 2,2,3-trisubstituted methylenecyclopropanes **3a**, **4a**, **3b**, **4b** and **16a**, **17a**, **16b**, **17b**

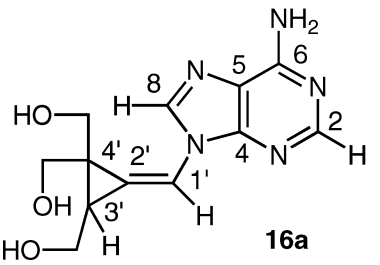
Compound[a]	$H_{1'}$	H_8	$C_{3'}$	$C_{4'}$
3a	7.37	8.82	11.7	31.4
4a	7.48	8.49	14.4	29.7
3b	7.07	8.41	11.5	31.3
4b	7.21	8.03	14.3	29.5
16a	7.44	8.79	26.3	35.1
17a	7.44	8.76	28.5	32.6
16b	7.15	8.38	26.2	35.0
17b	7.19	8.31	28.4	32.5

[a]CD_3SOCD_3 as solvent. For numbering of signals see Table 2. Values for **3a**, **4a**, **3b**, and **4b** were taken from Ref.[3]

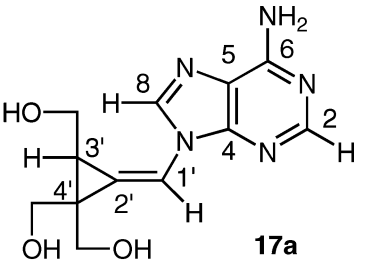
nucleosides.[1,2] Thus, the *Z(cis)* isomers are faster moving than *E(trans)* isomers. The purine H_8 of the *Z(cis)* isomers is more deshielded than that of *E(trans)* isomers whereas an opposite trend holds for the alkene $H_{1'}$. In the ^{13}C NMR spectra. The $C_{3'}$ signal of the *Z*-isomers is generally upfield relative to that of *E*-isomers but the opposite is true for the $C_{4'}$ chemical shift. With analogues **16** and **17** the situation is more complex. The chromatographic mobility trend $Z > E$ was preserved but differences between the chemical shifts of H_8 and $H_{1'}$ were significantly smaller than those of analogues **3** and **4** (Table 1). Thus, the $\Delta\delta H_8$ were only 0.03–0.07 for **16a**, **16b** and **17a**, **17b** whereas these values were[3] 0.33–0.38 for **3a**, **3b** and **4a**, **4b**. This may probably be caused by the fact that both *Z* and *E* isomers of the series **16** and **17** have hydroxymethyl groups located in the proximity of the H_8. An absence of significant differences between the $H_{1'}$ chemical shifts of the *Z*- and *E*-isomers **16** and **17** has little diagnostic value. In contrast, the chemical shift pattern of cyclopropane carbons observed previously for **3** and **4** $C_{3'}(Z) < C_{3'}(E)$ and $C_{4'}(Z) > C_{4'}(E)$ is preserved.

The final corroboration of the *Z/E* assignment came from the NOE experiments with adenine analogues **16a** and **17a** (Table 2). The NOE enhancements (0.72 and 1.76%) were observed between the $H_{1'}$ and $H_{3'}$ of the *Z*-isomer **16a** where both protons are in a *cis* arrangement but they were absent in the *E*-isomer **17a** where this orientation is *trans*. A strong interaction was also noted for **16a** between the H_8 of the purine residue in an anti-like conformation and hydroxy group protons of the geminal 4'-CH_2OH at δ 5.17 (0.21 and 3.34%) and 4.86 (1.39%). As expected, the NOE enhancement was seen between the *cis*-configured $H_{1'}$ and hydroxy group of the 3′-CH_2OH at δ 4.77 (0.99%). An interaction between the $H_{3'}$ and hydroxy group of the 3′-CH_2OH at δ 4.77 (1.98 and 1.52%) also was noticeable. Surprisingly, no NOE was observed between the H_8 and methylene protons of the CH_2OH groups in either isomer **16a** or **17a**.

TABLE 2 The NOE enhancements of relevant ^{1}H NMR signals of 2,2,3-tris(hydroxymethyl)-methylenecyclopropanes



16a



17a

Compound	H_{iir}	δ	H_{obs}	δ	NOE (%)
16a	$H_{1'}$	7.44	$H_{3'}$	1.91	0.72
	$H_{3'}$	1.91	$H_{1'}$	7.44	1.76
	H_8	8.79	4′-CH_2**OH**	5.17	0.21
	4′-CH_2**OH**	5.17	H_8	8.79	3.34
	4′-CH_2**OH**	4.86	H_8	8.79	1.39
	3′-CH_2**OH**	4.77	$H_{1'}$	7.44	0.99
	$H_{3'}$	1.91	3′-CH_2OH	4.77	1.52
	3′-CH_2OH	4.77	$H_{3'}$	1.91	1.98
17a	H_8	8.76	$H_{3'}$	2.08	0.94
	$H_{3'}$	2.08	H_8	8.76	1.40
	H_8	8.76	3′-CH_2**OH**	5.08	2.68
	3′-CH_2**OH**	5.08	H_8	8.76	1.44
	4′-CH_2**OH**	4.77	$H_{1'}$	7.44	1.40
	$H_{3'}$	2.08	3′-CH_2**OH**	5.08	1.54
	3′-CH_2**OH**	4.77	$H_{3'}$	2.08	0.59

Interactions between the H_8 and $H_{3'}$ (0.94 and 1.40%) absent in the Z-isomer **16a** were clearly in accord with the *E* configuration of the base in isomer **17a**. In addition, the NOE enhancements were observed between the hydroxy group of the 3′-CH_2OH at δ 5.17 facing H_8 (1.44 and 2.68%). No such interaction with other hydroxy groups was noted but it took place between the hydroxyl proton of the *cis*-configured 4′-CH_2OH at δ 4.75 and $H_{1'}$ (1.40%). Not surprisingly, the NOE enhancements were seen between the $H_{3'}$ and 3′-CH_2OH (1.54 and 0.59%). It is also noteworthy that the protons of hydroxy groups juxtaposed to purine H_8 (4′-CH_2OH of **16a** and 3′-CH_2OH of **17a**) in an *anti*-like conformation are the most deshielded ones.

Analogues **16a**, **16b**, **17a**, and **17b** were inactive against the following viruses: Human immunodeficiency virus type 1 (HIV-1), hepatitis B and C virus (HBV and HCV), herpes simplex virus 1 and 2 (HSV-1 and HSV-2), human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), respiratory syncytial virus A (RSV-A), adenovirus, measles, and

parainfluenza virus. Adenine analogues **16a** and **17a** were not substrates for adenosine deaminase.

EXPERIMENTAL SECTION

General Methods

The UV spectra were measured in ethanol and NMR spectra were determined at 300 or 400 MHz (^{1}H), 75 or 100 MHz (^{13}C) in CD_3SOCD_3 unless stated otherwise. Mass spectra were determined in electrospray ionization mode (ESI-MS, methanol–NaCl or methanol–KOAc). For abbreviations of common solvents, reagents and protecting groups see *J. Org. Chem.* 2005, *70*, 26A–27A.

Starting Materials

3-Methylenecyclopropane-*trans*-1,2-dicarboxylic acid (Feist's acid, **18a**) was prepared as described.[11]

Diethyl 3-Methylenecyclopropane-*trans*-1,2-dicarboxylate (18b). Conc. H_2SO_4 (1.0 mL) was added dropwise to a stirred mixture of Feist's acid (**18a**, 14.2 g, 0.1 mol) and ethanol (200 mL). The resultant solution was refluxed for 24 hours. Ethanol was evaporated in vacuo, ether (200 mL) was added, the organic phase was washed with aqueous $NaHCO_3$ (5%, 3 × 40 mL) and it was dried ($MgSO_4$). The solvent was removed and the residue was chromatographed using hexanes-ether (30 : 1 to 20 : 1) to give ester **18b** (18.41 g, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.18 (m, 6H, CH_3), 2.77 (m, 2H, H_1, H_2), 4.07 (m, 4H, CH_2 of Et), 5.57 (m, 2H, CH_2=). ^{13}C NMR 14.2 (CH_3), 25.9 (C_1, C_2), 61.4 (CH_2 of Et), 106.4 (CH_2=), 129.3 (C_3), 169.4 (C=O).

Diethyl 1-Hydroxymethyl-3-methylenecyclopropane-*cis*-1,2-dicarboxylate (19). Ester **18b** (8.0 g, 40 mmol) was dissolved in THF (160 mL), dry LiCl (10.2 g, 0.24 mol) was added, the mixture was cooled to -78°C and LDA in THF (1.8 M, 15.65 mL, 38 mmol)[5,12] was added dropwise with stirring under N_2. The stirring was continued for 40 minutes whereupon gaseous CH_2O generated from paraformaldehyde (4.8 g, 0.12 mol) by heating at 180–200°C was introduced into the reaction mixture. After 30 minutes, the reaction was quenched with HCl (5%, 100 mL). THF was evaporated, the mixture was extracted with ether (200 mL), the organic phase was successively washed with HCl (5%, 2 × 50 mL), aqueous $NaHCO_3$ (5%, 3 × 50 mL) and it was dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on a silica gel column using hexanes-ether (10 : 1 to 2 : 1) to give product **19** (3.81 g, 44%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.19 (m, 6H, CH_3), 2.59 (m, 1H, H_1), 3.12 (bs, 1H, OH), 3.65, 3.79 (AB, 2H, J_{AB} = 12.2 Hz, CH_2O), 4.08 (m, 4H, CH_2 of Et), 5.67 (d, 1H,

J = 1.6 Hz), 5.69 (d, 1H, J = 2.4 Hz, CH_2=). ^{13}C NMR 14.1, 14.3 (CH_3), 29.2 (C_1), 36.7 (C_2), 61.5, 61.8 (CH_2 of Et), 65.2 (CH_2O), 107.2 (CH_2=), 130.2 (C_3), 168.0, 169.5 (C=O). ESI-MS 229 (M + H, 67.9), 251 (M + Na, 100.0), 479 (2M + Na, 32.7).

1,1,2-Tris(acetoxymethyl)-3-methylenecyclopropane (22). A solution of compound **19** (3.50 g, 15.3 mmol) in ether (42 mL) was added to a stirred suspension of $LiAlH_4$ (1.75 g, 46.1 mmol) in ether (30 mL) at such a rate to maintain a gentle reflux. The resultant mixture was refluxed for 6 hours. It was then quenched carefully with water (2 mL) and NaOH (10%, 6 mL). The solvents were evaporated in vacuo and the solid residue was extracted in a Soxhlet apparatus with methanol (80 mL) by refluxing for 11 hours. The extract was dried over $MgSO_4$ and methanol was evaporated. The crude product was dried at 2 mm torr and room temperature to give triol **21** (1.82 g, 82%) which was directly acetylated as follows. A mixture of **21** (1.80 g, 12.5 mmol), acetic anhydride (7 mL) and pyridine (3 mL) was stirred at room temperature for 7 hours. Water was then added and the reaction was quenched with water, and the product was extracted with cold (4°C) pentane (50 mL). The combined organic phase was washed successively with saturated aqueous $CuSO_4$, HCl (5%), aqueous $NaHCO_3$, and brine. It was then dried with $MgSO_4$, the solvent was evaporated, and the residue was chromatographed on a silica gel column in hexanes-ether (10 : 1 to 5 : 1) to give triacetate **22** as a colorless liquid (2.96 g, 88%). ^{1}H NMR ($CDCl_3$) δ 2.00, 2.01 (2s overlapped with m, 10H, CH_3, H_1), 3.91–3.96 (m, 2H), 4.07, 4.11 (AB, 2H, J_{AB} = 12.2 Hz), 4.18–4.26 (m, 2H, CH_2O), 5.46 (d, 1H, J = 1.6 Hz), 5.50 (d, 1H, J = 2.4 Hz, CH_2=). ^{13}C NMR 21.0, 21.1 (CH_3), 24.7 (C_1), 27.2 (C_2), 62.2, 62.6, 66.8 (CH_2OAc), 107.1 (CH_2=), 135.1 (C_3), 170.88, 170.90, 171.0 (C=O). ESI-MS (MeOH + KOAc) 309 (M + K, 100.0).

***cis, trans*-3-Bromo-3-bromomethyl-1,1,2-tris(acetoxymethyl)cyclopropane (23)**. Pyridinium tribromide (1.7 g, 90%, 4.81 mmol) was added to a solution of compound **22** (0.96 g, 3.56 mmol) in CH_2Cl_2 (15 mL) at -20°C with stirring. The reaction mixture was stirred for 1 hour and then at room temperature for 2 hours. Ethyl acetate (100 mL) was added and the organic phase was washed with a saturated aqueous solution of $Na_2S_2O_3$, $NaHCO_3$ and water whereupon it was dried ($MgSO_4$). The solvents were evaporated and the residue was chromatographed on a silica gel column using hexanes-ethyl acetate (3 : 1 to 1 : 1) to give the product **23** (1.37 g, 90%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.04, 2.05, 2.06 (3s overlapped with m, 10H, CH_3, H_1), 3.79, 3.91 (AB, J_{AB} = 10.0 Hz), 3.85, 3.89 (AB, 2H, J_{AB} = 12.6 Hz, CH_2Br), 4.08–4.48 (m, 6H, CH_2OAc). ESI-MS 451 (53.0), 453 (100.0), 455 (M + Na, 48.8).

(*Z,E*)-9-{[1,2,2-tris(acetoxymethyl)cyclopropylidene]methyl}adenine (24a). A mixture of adenine (330 mg, 2.45 mmol), dibromide **23** (0.96 g, 2.23 mmol) and flame-dried K_2CO_3 (1.85 g, 13.9 mmol) was stirred in DMF (15 mL) at 100°C under N_2 for 2.5 hours. After cooling, the insoluble

portion was filtered off and it was washed with DMF. The filtrate was concentrated in vacuo and the residue was chromatographed on a silica gel column using EtOAc-MeOH (100 : 0 to 20 : 1) to give a mixture of *E*- and *Z*-isomers **24a** (521 mg, 58%) in the ratio of 1.6 : 1 as a white solid, m.p. 167–169°C. UV λ_{max} (EtOH), 279 nm (ε 8,200), 264 (ε 11,400), 229 (ε 22,100). ^{1}H NMR ($CDCl_3$) δ 2.06, 2.08, 2.10, 2.11 (4s, 9H, CH_3), 2.30, 2.47 (2t, J = 6.8 Hz, $H_{3'}$), 4.02–4.54 (m, 6H, CH_2OAc), 5.98, 6.02 (2s, 2H, NH_2), 7.62, 7.66 (2d, 1H, J = 1.6 Hz, $H_{1'}$), 8.33, 8.39, 8.42 (3s, 2H, H_2, H_8). ^{13}C NMR 21.0–21.1 (4 partially overlapped s, CH_3), 23.8, 26.2, 27.0, 29.2 ($C_{3'}$, $C_{4'}$), 62.0, 62.2, 62.3, 62.5, 66.5, 66.8 (CH_2O), 113.9 (2s), 116.0, 116.3, 119.5 ($C_{1'}$, $C_{2'}$, C_5), 137.7, 138.0 (C_8), 148.6, 149.1 (C_4), 153.4 (C_2), 155.5 (C_6), 170.6, 170.7, 170.8, 170.9 (C = O). ESI-MS 404 (M + H, 100.0), 426 (M + Na, 78.3).

(*Z*)- and (*E*)-9-{[1,2,2-tri(hydroxymethyl)cyclopropylidene]methyl}adenine (16a) and (17a). A mixture of the *Z,E*-isomers **24a** (400 mg, 0.99 mmol) and K_2CO_3 (410 mg, 2.97 mmol) in methanol (50 mL) was stirred at room temperature for 30 minutes. After removal of methanol, the residue was chromatographed in CH_2Cl_2-methanol (10 : 1 to 5 : 1) to give the faster moving *Z*-isomer **16a** (130 mg, 47%) followed by *E*-isomer **17a** (106 mg, 39%).

Z-isomer **16a**: M.p. 255–257°C. UV λ_{max} (EtOH) 279 nm (ε 7,700), 264 (ε 10,300), 229 (ε 23,400). ^{1}H NMR (DMSO-d_6) δ 1.91 (t, 1H, J = 6.8 Hz, $H_{3'}$), 3.32 (m, overlapped with H_2O), 3.53 (m, 2H), 3.60 (m, 1H), 3.85 (dd, 1H, J = 11.4, 6.6 Hz), 3.93 (dd, 1H, J = 10.6, 5.0 Hz, CH_2O), 4.77 (t, 1H, J = 5.4 Hz, 3′-CH_2**OH**), 4.87 (t, J = 4.4 Hz), 5.17 (t, 1H, J = 4.8 Hz, 4′-CH_2**OH**), 7.32 (s, 2H, NH_2), 7.44 (s, 1H, $H_{1'}$), 8.16 (s, 1H, H_2), 8.79 (s, 1H, H_8). ^{13}C-NMR 26.3 ($C_{3'}$), 35.1 ($C_{4'}$), 59.2, 60.1, 64.1 (CH_2O), 111.2 ($C_{1'}$), 119.1, 120.9 ($C_{2'}$, C_5), 138.3 (C_8), 148.6 (C_4), 153.6 (C_2), 156.7 (C_6). ESI-MS 278 (M + H, 100.0), 300 (M + Na, 56.6), 577 (2M + Na, 21.4). Anal. Calcd for $C_{12}H_{15}N_5O_3$: C, 51.98; H, 5.45; N: 25.26. Found: C, 51.89; H, 5.58; N, 25.11.

E-isomer **17a**: M.p. 248–249°C. UV λ_{max} (EtOH) 279 nm (ε 7,300), 265 (ε 9,800), 229 (ε 21,900). ^{1}H NMR (DMSO-d_6) δ 2.08 (t, 1H, J = 6.0 Hz, $H_{3'}$), 3.30 (m, overlapped with H_2O, OCH_2), 3.45 (dd, 1H, J = 11.2, 4.8 Hz, OCH_2), 3.58–3.78 (cluster of m, 4H, OCH_2), 4.68 (t, 1H, J = 5.2 Hz, OH), 4.74 (t, J = 5.6 Hz, 4′-CH_2**OH**), 5.08 (t, 1H, J = 4.0 Hz, 3′-CH_2**OH**), 7.33 (s, 2H, NH_2), 7.44 (s, 1H, $H_{1'}$), 8.16 (s, 1H, H_2), 8.76 (s, 1H, H_8). ^{13}C NMR 28.5 ($C_{3'}$), 32.6 ($C_{4'}$), 59.7, 59.9, 64.3 (CH_2O), 110.8 ($C_{1'}$), 119.1, 121.2 ($C_{2'}$, C_5), 138.2 (C_8), 148.7 (C_4), 153.7 (C_2), 156.7 (C_6). ESI-MS 278 (M + H, 100.0), 300 (M + Na, 43.2), 577 (2M + Na, 16.0). Anal. Calcd for $C_{12}H_{15}N_5O_3$:C, 51.98; H, 5.45, N, 25.26. Found: C, 51.72; H, 5.59; N, 25.08.

(*Z,E*)-2-Amino-6-chloro-9-{[1,2,2-tri(acetoxymethyl)cyclopropylidene]methyl}purine (24b). A mixture of 2-amino-6-chloropurine (0.75 g, 4.44 mmol), dibromide **23** (1.72 g, 4.0 mmol), and K_2CO_3 (3.32 g, 24 mmol) in DMF (20 mL) was stirred at room temperature for 48 hours and at

100–105°C for 3.5 hours. After cooling, the insoluble portion was filtered off and washed with DMF, and the filtrate was evaporated. The crude product was chromatographed in hexanes-AcOEt (2 : 1 to 100% AcOEt) to give a mixture of *Z*- and *E*-isomers **24b** (1.18 g, 67%) in the ratio of 1.4 : 1.0 as a white solid, m.p. 171–172°C. UV λ_{max} (EtOH) 311 nm (ε 10,900), 231 (ε 39,500). ^{1}H NMR ($CDCl_3$) δ 2.04, 2.06, 2.08, 2.086, 2.09, 2.10 (6s, 9H, CH_3), 2.26, 2.46 (2dt, 1H, J = 7.2, 2.4 Hz, $H_{3'}$), 3.99–4.51 (cluster of m, 6H, CH_2O), 5.50 (bs, 2H, NH_2), 7.39, 7.41 (2d, 1H, J = 1.6 Hz, $H_{1'}$), 8.15, 8.29 (2s, 1H, H_8). ^{13}C NMR 23.9, 27.1 ($C_{3'}$), 27.2, 29.4 ($C_{4'}$), 61.9, 62.2, 62.5, 62.8, 66.6, 67.0 (CH_2O), 113.6, 113.9 ($C_{1'}$), 116.6, 116.7, 125.2, 125.3 ($C_{2'}$, C_5), 139.7 (C_8), 151.86, 151.88 (C_4), 152.5, 152.6 (C_2), 159.7, 159.8 (C_6), 170.71, 170.73, 170.86, 170.9, 171.0 (C=O). ESI-MS 438, 440 (M + H, 94.7, 29.6), 460, 462 (M + Na, 100.0, 33.1), 897, 899 (2M + Na, 18.3, 13.0).

(*Z*)- and (*E*)-2-Amino-6-chloro-9-{[1,2,2-tri(hydroxymethyl)cyclopropylidene]methyl}-purine (16c) and (17c). A mixture of *Z*- and *E*-isomers **24b** (1.1 g, 2.51 mmol) was dissolved in 10% NH_3 in MeOH (80 mL) at 0°C and the mixture was stirred for 8 hours at room temperature. The volatile components were evaporated and the residue was chromatographed on a silica gel column in AcOEt-methanol (100 : 0 to 10 : 1) to give the faster moving *Z*-isomer (340 mg, 44%) followed by *E*-isomer (210 mg, 27%).

Z-isomer **16c**: M.p. 222–224°C. UV λ_{max} (EtOH) 310 nm (ε 8,100), 236 (ε 31,700). ^{1}H NMR (DMSO-d_6) δ 1.91 (t, 1H, J = 7.2 Hz, $H_{3'}$), 3.29 (dd, 1H, J = 10.4, 5.6 Hz), 3.48–3.61 (2m, 3H), 3.81 (dd, 1H, J = 11.6, 6.0 Hz), 3.92 (dd, 1H, J = 10.6, 5.0 Hz, CH_2O), 4.77 (t, 1H, J = 5.4 Hz), 4.85 (t, J = 5.0 Hz), 5.15 (t, 1H, J = 5.0 Hz, OH), 7.01 (s, 2H, NH_2), 7.26 (s, 1H, $H_{1'}$), 8.78 (s, 1H, H_8) ^{13}C NMR 26.4 ($C_{3'}$), 35.1 ($C_{4'}$), 59.1, 59.9, 64.0 (CH_2O), 110.7 ($C_{1'}$), 121.7, 123.8 ($C_{2'}$, C_5), 140.4 (C_8), 150.2 (C_4), 153.0 (C_2), 160.7 (C_6). ESI-MS 312, 314 (M + H, 100.0, 30.5), 334, 336 (M + Na, 16.9, 5.0). Anal. Calcd for $C_{12}H_{14}ClN_5O_3Cl \times 0.3\ H_2O$: C, 45.45; H, 4.64; N, 21.89. Found: C, 45.57; H, 4.67; N, 21.87.

E-isomer **17c**: M.p. 220–222°C (dec.). UV λ_{max} (EtOH) 310 nm (ε 8,200), 236 (ε 32,300). ^{1}H NMR (DMSO-d_6) δ 2.08 (t, 1H, J = 6.4 Hz, $H_{3'}$), 3.29 (dd, 1H, J = 10.4, 5.6 Hz), 3.45 (dd, 1H, J = 11.2, 4.8 Hz), 3.53–3.60 (m, 1H), 3.65–3.73 (m, 2H), 3.76–3.81 (m, 1H, CH_2O), 4.68 (t, 1H, J = 5.2 Hz), 4.75 (t, J = 5.6 Hz), 5.04 (t, 1H, J = 4.8 Hz, OH), 7.00 (s, 2H, NH_2), 7.28 (d, 1H, J = 1.6 Hz, $H_{1'}$), 8.73 (s, 1H, H_8). ^{13}C NMR 28.5 ($C_{3'}$), 32.8 ($C_{4'}$), 59.7, 59.8, 64.3 (CH_2O), 110.4 ($C_{1'}$), 122.0, 123.8 ($C_{2'}$, C_5), 140.4 (C_8), 150.2 (C_4), 153.1 (C_2), 160.7 (C_6). ESI-MS 312, 314 (M + H, 100.0, 33.6), 334, 336 (M + Na, 16.4, 5.7). Anal. Calcd for $C_{12}H_{14}N_5O_3Cl$ x 0.25 H_2O: C, 45.58; H, 4.62; N, 22.15. Found: C, 45.52; H, 4.61; N, 21.92.

(*Z*)-9-{[1,2,2-tri(hydroxymethyl)cyclopropylidene]methyl}guanine (16b). A solution of the *Z*-isomer **16c** (120 mg, 0.39 mmol) in formic acid (80%, 6 mL) was heated at 80°C with stirring for 4 hours. After cooling, formic acid was evaporated in vacuo and the crude product was dissolved in methanol

(30 mL). The precipitated white solid was filtered off and it was stirred with NH_3 in methanol (20%, 20 mL) at 0°C for 1 hour. The volatile components were evaporated and the crude product was recrystallized for NH_4OH (28%)[14] to give the title compound **16b** (98 mg, 87%), m.p. >300°C. UV λ_{max} (EtOH) 271 nm (ε 11,100), 231 (ε 21,500). ^{1}H NMR (DMSO-d_6) δ 1.86 (t, 1H, J = 7.2 Hz, $H_{3'}$), 3.25–3.33 (m partly overlapped with H_2O), 3.45 (dd, 1H, J = 11.2, 3.2 Hz), 3.51–3.59 (m, 2H), 3.82 (dd, 1H, J = 11.2, 6.4 Hz), 3.91 (dd, 1H, J = 10.4, 4.0 Hz, CH_2O), 4.75 (t, 1H, J = 5.6 Hz), 4.81 (t, J = 4.8 Hz,), 5.11 (t, 1H, J = 4.8 Hz, OH), 6.50 (s, 2H, NH_2), 7.15 (s, 1H, $H_{1'}$), 8.38 (s, 1H, H_8), 10.64 (s, 1H, NH). ^{13}C NMR 26.2 ($C_{3'}$), 35.0 ($C_{4'}$), 59.1, 60.1, 64.0 (CH_2O), 111.0 ($C_{1'}$), 116.9, 120.5 ($C_{2'}$, C_5), 135.7 (C_8), 150.4 (C_4), 154.6 (C_2), 157.4 (C_6). ESI-MS (MeOH + KOAc) 332 (M + K, 100.0), 625 (2M + K, 18.2). Anal. Calcd for $C_{12}H_{15}N_5O_4$ x H_2O: C, 46.30; H, 5.50; N, 22.50. Found: C, 46.72; H, 5.17; N, 22.17.

(*E*)-9-{[1,2,2-tri(hydroxymethyl)cyclopropylidene]methyl}guanine (17b). The procedure described for the *Z*-isomer **16b** was used with compound **17c** (115 mg, 0.37 mmol) as a starting material to give the *E*-isomer **17b** (89 mg, 83%), m.p. >300°C. UV λ_{max} (EtOH) 272 nm (ε 10,900), 232 (ε 19,800). ^{1}H NMR (DMSO-d_6) δ 2.02 (t, 1H, J = 7.2 Hz, $H_{3'}$), 3.26 (dd, 1H, J = 10.4, 4.8 Hz,), 3.43 (dd, 1H, J = 10.4, 4.8 Hz), 3.55–3.61 (m, 1H), 3.55–3.74 (m, 3H, CH_2O), 4.65 (bs, 1H), 4.73 (t, J = 4.8 Hz), 5.00 (s, 1H, OH), 6.50 (s, 2H, NH_2), 7.19 (s, 1H, $H_{1'}$), 8.31 (s, 1H, H_8), 10.68 (s, 1H, NH). ^{13}C NMR 28.4 ($C_{3'}$), 32.5 ($C_{4'}$), 59.7, 59.8, 64.4 (CH_2O), 110.8 ($C_{1'}$), 116.9, 120.6 ($C_{2'}$, C_5), 135.7 (C_8), 150.4 (C_4), 154.6 (C_2), 157.4 (C_6). ESI-MS 332.0 (M + K, 100.0), 625 (2M + K, 29.2). Anal. Calcd for $C_{12}H_{15}N_5O_4$ x H_2O: C, 46.30; H, 5.50; N, 22.50. Found: C, 46.55; H, 5.26; N, 22.15.

REFERENCES AND NOTES

1. Zemlicka, J. Unusual analogues of nucleosides: Chemistry and biological activity. *In* Recent Advances in Nucleosides: Chemistry and Chemotherapy. Chu, C.K., Ed., Elsevier: Amsterdam, 2002, 327–357.
2. Zemlicka, J.; Chen, X. Methylenecyclopropane analogs of nucleosides as antiviral agents. *In* Frontiers in Nucleosides and Nucleic Acids. Schinazi, R.F.; Liotta, D.C., Eds., IHL Press: Tucker, Georgia, 2004, 267–307.
3. 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.
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. Zhou, S.; Kern, E.R.; Gullen, E.; Cheng, Y.-C.; Drach, J.C.; Matsumi, S.; Mitsuya, H.; Zemlicka, J. (*Z*)- and (*E*)-[2-Fluoro-(hydroxymethyl)cyclopropylidene]methylpurines and pyrimidines, a new class of methylenecyclopropane analogues of nucleosides: Synthesis and antiviral activity. *J. Med. Chem.* **2004**, 47, 6964–6972.
6. Zhou, S.; 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.

7. Zhou, S.; Zemlicka, J.; Kern, E.R.; Drach, J.C. Fluoroanalogues of anti-cytomegalovirus agent cyclopropavir: Synthesis and antiviral activity of (*E*)- and (*Z*)-9-{[2,2-bis(hydroxymethyl)-3-fluorocyclopropylidene]methyl}adenines and -guanines. *Nucleosides, Nucleotides Nucleic Acids* in press.
8. Cheng, C.; Shimo, T.; Somekawa, K.; Kawaminami, M. Reactions of methylenecyclopropanes with diethylzinc-bromoform system, and the utilization for synthesis of a novel cyclopropylidene-nucleoside. *Tetrahedron Lett.* **1997**, 38, 9005–9008.
9. Chen, X.; Matsumi, S.; Mitsuya, H.; Zemlicka, J. Synthesis of (*Z*)-(2,3-bis-hydroxymethyl)methylene-cyclopropane analogues of purine nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2003**, 22, 265–274.
10. Choi, B.G.; Kwak, E.Y.; Hong, J.H.; Lee, C.K. Synthesis and antiviral activity of novel exomethylene cyclopropyl nucleosides. *Nucleosides, Nucleotides Nucleic Acids* **2001**, 20, 1059–1062.
11. Gilchrist, T.L.; Reese, C.W. Synthesis of 3-bromo-2-pyrones and their reactions with bases. *J. Chem. Soc. (C)* **1968**, 769–775.
12. Zhou, S.; Zemlicka, J. A new alkylation-elimination method for synthesis of antiviral fluoromethylenecyclopropane analogues of nucleosides. *Tetrahedron* **2005**, 61, 7112–7116.
13. A base-catalyzed isomerization of Feist's acid accompanied by anhydride formation was described: Ettlinger, M. G.; Kennedy, F. The *cis*-isomer of Feist's acid. *Chem & Ind.* **1957**, 891.
14. Compound **16b** is more soluble in NH_4OH than in water. Some NH_3 was lost during the process.