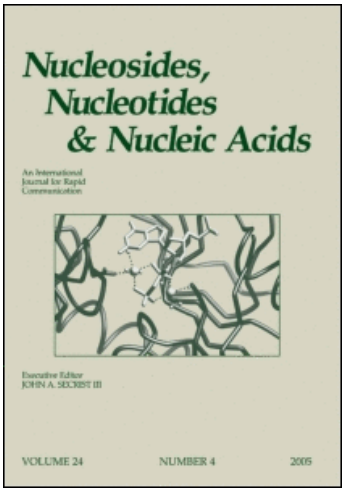

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Synthesis of (*Z*)- and (*E*)-9-[(2-Hydroxyethylidene)cyclopropyl]adenine—New Methylenecyclopropane Analogues of Adenosine and Their Substrate Activity for Adenosine Deaminase

Yao-Ling Qiu[a]; Mohamad B. Ksebati[b]; Jiri Zemlicka[a]
[a] Department of Chemistry, Experimental and Clinical Chemotherapy Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI [b] Central Instrumentation Facility, Department of Chemistry, Wayne State University, Detroit, MI

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SYNTHESIS OF *(Z)*- AND *(E)*-9-[(2-HYDROXYETHYLIDENE)-CYCLOPROPYL]ADENINE - NEW METHYLENECYCLOPROPANE ANALOGUES OF ADENOSINE AND THEIR SUBSTRATE ACTIVITY FOR ADENOSINE DEAMINASE#

Yao-Ling Qiu[1], Mohamad B. Ksebati[2] and Jiri Zemlicka*,[1]

[1]Department of Chemistry, Experimental and Clinical Chemotherapy Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201-1379

[2]Central Instrumentation Facility, Department of Chemistry, Wayne State University, Detroit, MI 48202

Dedicated to the memory of Dr. Gertrude B. Elion

ABSTRACT. Synthesis of *Z*- and *E*-methylenecyclopropane analogues of adenosine **3** and **4** by alkylation of adenine with novel alkylating agent **5** is described. The *E*-isomer **4** is a substrate for adenosine deaminase. Compounds **3** and **4** were tested for antiviral activity against HCMV, HSV-1, HSV-2, EBV, VZV, HBV and HIV-1.

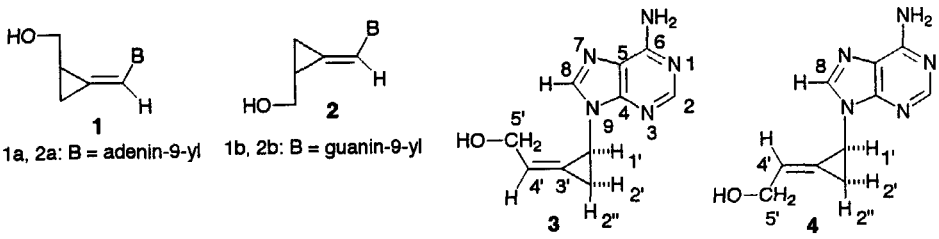
Recently, we described a new class of nucleoside analogues in which the ribofuranoside moiety is replaced with a methylenecyclopropane function.[1-8] Purine derivatives such as synadenol (**1a**) and synguanol (**1b**) exhibit potent antiviral activity, particularly against human cytomegalovirus (HCMV). The corresponding *E*-isomers **2a** and **2b** are much less potent. In order to define the scope and limitations of biological activity in this series it is necessary to investigate other methylenecyclopropane nucleoside analogues.

The methylenecyclopropane system can be regarded as a rigid linker between the two functions essential for antiviral effect: base residue and the hydroxymethyl group. For this reason we became interested in analogues of the type of **3** and **4** derived by a simple interchange of both functions in **1a** and **2a**. An alkylation-elimination approach to both **3** and **4** was regarded as the most convenient, provided that an appropriate

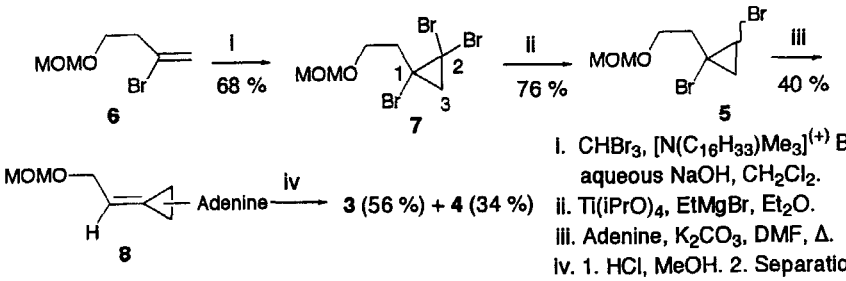
alkylating agent can be obtained by a simple procedure. Also, such a reagent can also be useful for alkylation of other heterocyclic systems. In this communication, we describe the synthesis of analogues **3** and **4** using a new alkylating reagent **5**.

RESULTS AND DISCUSSION

3-Bromo-3-buten-1-ol was protected with the methoxymethyl (MOM) group[9] to give compound **6** (98 %). Addition of dibromocarbene generated from $CHBr_3$ under phase-transfer conditions[10] led to tribromo derivative **7** (68 %, Scheme 1). Partial



Scheme 1



reduction of one of the geminal bromine atoms using titanium 2-propoxide and ethylmagnesium bromide[11] furnished the desired alkylating reagent **5** as a 3 : 1 isomeric mixture (76 %). Alkylation-elimination with adenine using K_2CO_3 in DMF at 120°C for 15 h gave the MOM-protected *Z,E*-intermediates **8** (39.5 %). Deprotection with 0.3 M HCl in methanol afforded, after separation by column chromatography on silica gel, the desired *Z*- and *E*-isomers of methylenecyclopropane analogues **3** and **4** in 56 and 34 % yield, respectively. The UV spectra (UV_{max} 260 nm) indicated that both compounds are N^9-alkylated adenines.[12]

The *Z,E*-isomeric assignment was performed as follows. The chemical shifts of purine H8 which were assigned by (H,C) COSY spectra were almost identical in both *Z*- and *E*-isomers (**3**, **4**) in contrast to analogues[1] **1a** and **2a**. Chemical shifts of the olefinic protons H4' and OH followed the pattern observed previously[1] for **1a** and **2a**. Along

Table 1. Selected NOE enhancements of analogues **3** and **4**.

Z-Isomer **3**			*E*-Isomer **4**		
Irradiated	Observed	% NOE	Irradiated	Observed	% NOE
H8	H5'	1.4	H8	H4'	2.5
H8	OH	0.5	H4'	H8	3.3
H5'	H8	2.3			
OH	H8	3.5			

with polarity considerations /the *Z*-(cis) isomers are more chromatographically mobile than *E*-(trans) isomers[1]/ it was then possible to assign tentatively the *Z*-configuration to compound **3**. This assignment was confirmed by NOE experiments (Table 1). As expected, the NOE enhancements observed between the H8 and H5' as well as H8 and OH are in accord with the *Z*-isomeric structure of **3**. By contrast, NOE between the H4' and H8 is characteristic for the *E*-isomer **4**.

Analogues **3** and **4** were tested against the following viruses: HCMV, HSV-1, HSV-2, EBV, VZV, HBV and HIV-1. The details of the assays were described previously[1]. In contrast to synadenol (**1a**), only the *Z*-isomer **3** exhibited a moderate effect against EBV in H-1 cells (EC_{50} 36 μM, CC_{50} >50 μM) but it was inactive in Daudi cells. Both analogues **3** and **4** were ineffective against the rest of the viruses at the highest concentration tested (100 μM, EBV/H-1 50 μM and HBV 10 μM) probably because of a lack of substrate activity toward viral or intracellular nucleoside kinases (5'-nucleotidases).

The *E*-isomer **4** is a substrate for adenosine deaminase. Thus, 82 % of **4** was deaminated after 45 h whereas the *Z*-isomer **3** remained intact. A similar trend was observed in methylenecyclopropane analogues[1] **1a** and **2a** and, generally, in all unsaturated *Z,E* (*cis, trans*) analogues of adenosine examined to date.[13-15]

EXPERIMENTAL SECTION

General Methods. See[1]. The NMR spectra were determined at 300 or 400 MHz (^{1}H) and 75 or 100 MHz (^{13}C) in CD_3SOCD_3 unless stated otherwise.

1-(2-Methoxymethoxyethyl)-1,2,2-tribromocyclopropane (7). A warm solution of NaOH (48.0 g, 1.20 mol) in water (32 mL) was added dropwise over 15

min into a mixture of **6** (25.32 g, 130 mmol), cetyltrimethyl ammonium bromide (1.42 g, 3.90 mmol) and $CHBr_3$ (98.6 g, 390 mmol) in CH_2Cl_2 (60 mL) with stirring and ice-cooling. The mixture was stirred for 18 h at room temperature and then it was partitioned between petroleum ether and water. The insoluble portion was filtered off, organic phase was washed with HCl (1 M), water, saturated $NaHCO_3$, water and brine. After evaporation of the solvents, ethanol was repeatedly evaporated from the residue. Water (50 mL) and $KMnO_4$ (5 g) were added and the mixture was worked up after addition of petroleum ether as described above to give crude product which was chromatographed on a silica gel column using hexane - ethyl acetate (98.5 : 1.5 → 95 : 5) furnishing a yellow oil of **7** (32.44 g, 68 %). ^1H NMR ($CDCl_3$) δ 1.97 (s, 2 H, H_3), 2.37 (td, 2 H, ^3J = 6.6 Hz, J = 1.2 Hz, 1-CH_2), 3.38 (s, 3 H, OCH_3), 3.90 (td, 2 H, ^3J = 6.6 Hz, J = 1.5 Hz, CH_2O), 4.64 (s, 2 H, OCH_2O); ^{13}C NMR 32.40 (C_1), 37.86 (C_3), 41.15 (1-CH_2), 42.48 (C_2), 55.31 (OCH_3), 66.02 (CH_2O), 96.54 (OCH_2O); HRMS calcd for $C_6H_8{}^{79}Br_3O$ (M - OCH_3) 332.8125. Found 332.8117. For $C_7H_{11}Br_3O_2$ calcd C, 23.09; H, 3.05; Br, 65.07. Found: C, 23.21; H, 3.11; Br, 64.86.

(*Z*)- and (*E*)-1,2-Dibromo-1-(2-methoxymethoxyethyl)cyclopropane (5). A solution of EtMgBr (3 M in ether, 20.57 mL, 61.7 mmol) diluted with ether (20 mL) was added dropwise during 1.5 h into a mixture of tribromide **7** (22.64 g, 61.7 mmol) and titanium (IV) 2-propoxide (920 μL, 3.09 mmol) in ether (100 mL) under N_2 with stirring at room temperature. After 10 min, the reaction was quenched by a dropwise addition of water (50 mL). The stirring was continued for another 10 min before adddition of petroleum ether (100 mL). The organic phase was washed successively with H_2SO_4 (10 %), water, saturated $NaHCO_3$, water and brine. The solvents were evaporated and the residue was distilled to give compound **5** as a yellow oil (13.54 g, 76.2 %), bp 100-105°C/2.8 Torr. The ^1H NMR indicated a 3 : 1 isomeric mixture containing 5-10 % of impurities. Both isomers were separated by chromatography on silica gel using hexane - ethyl acetate (99 : 1 → 95 : 5) but their mixture was used in the next step for alkylation of adenine. Minor (slower moving) isomer: ^1H NMR ($CDCl_3$) δ 1.28 (dd, 1 H, ^2J = 7.5 Hz, $^3J_{trans}$ = 6.3 Hz) and 1.56 (t, 1 H, $^2J = {}^3J_{cis}$ = 8.25 Hz, H_3), 2.03 (AB x t, 2 H, J_{AB} = 11.7 Hz, ^3J = 6.3 Hz, 1-CH_2), 2.95 (dd, 1 H, $^3J_{cis}$ = 8.4 Hz, $^3J_{trans}$ = 6.0 Hz, H_2), 3.37 (s, 3 H, OCH_3), 3.75 (t, 2 H, ^3J = 6.9 Hz, CH_2O), 4.61 (s, 2 H, OCH_2O); ^{13}C NMR 25.60, 26.19 (C_3, C_2), 37.87 (C_1), 41.44 (1-CH_2), 55.19 (OCH_3), 65.56 (CH_2O), 96.53 (OCH_2O); HRMS calcd for $C_7H_{12}{}^{79}BrO_2$ (M - Br) 207.0020. Found 207.0025. Major (faster moving) isomer: ^1H NMR ($CDCl_3$) δ 1.23 (dd, 1 H, ^2J = 8.25 Hz, $^3J_{trans}$ = 5.55 Hz) and 1.56 (t, 1 H, $^2J = {}^3J_{cis}$ = 8.4 Hz, H_3), 2.22 (t, 2 H, ^3J = 6.75 Hz, 1-CH_2), 3.37 (s, 3 H, OCH_3), 3.49 (dd, 1 H, $^3J_{cis}$ = 8.7 Hz, $^3J_{trans}$ = 5.7 Hz, H_2), 3.84 (t, 2 H, ^3J = 6.9 Hz, CH_2O), 4.64 (s, 2 H,

OCH_2O); ^{13}C NMR 26.17, 27.73 (C_3, C_2), 33.02 (C_1), 38.57 (1-CH_2), 55.22 (OCH_3), 65.50 (CH_2O), 96.49 (OCH_2O); HRMS calcd for $C_7H_{12}{}^{79}BrO_2$ (M - Br) 207.0020. Found 207.0021.

(*Z*)- and (*E*)-9-[(2-Methoxymethoxyethylidene)cyclopropyl]adenine (8). A mixture of adenine (1.35 g, 10 mmol), compound **5** (3.74 g, 13 mmol) and potassium carbonate (6.91 g, 50 mmol) in DMF (50 mL) was stirred under N_2 at room temperature. The temperature was gradually raised to 100°C. After 40 min, the temperature was increased to 120°C and the heating was continued for 15 h. After cooling, the insoluble portion was filtered off and the filtrate was evaporated. The residue was chromatographed on silica gel using CH_2Cl_2 - MeOH (95 : 5 → 9 : 1) as eluents to give an orange solid (1.03 g, 39.5 %). The ^1H NMR spectrum showed it was a mixture of *Z/E* isomers **8** in a ratio of 1.5 : 1 along with some minor impurities. ^1H NMR Z-Isomer: δ 1.72 (d, 1 H, ^2J = 10.8 Hz, $H_{2''}$) and 1.98 (t, 1 H, $^2J = {}^3J_{cis}$ = 9.15 Hz, $H_{2'}$), 3.21 (s, 3 H, OCH_3), 4.08 (m, 1 H, $H_{1'}$), 4.16 (d, 2 H, ^3J = 5.7 Hz, $H_{5'}$), 4.56 (s, 2 H, OCH_2O), 6.59 (brs, 1 H, $H_{4'}$), 7.26 (s, 2 H, NH_2), 8.03 (s, 1 H, H_8) and 8.15 (s, 1 H, H_2 of adenine). *E*-Isomer: δ 1.76 (d, 1 H, ^2J = 10.8 Hz, $H_{2''}$) and 1.94 (t, 1 H, $^2J = {}^3J_{cis}$ = 9.3 Hz, $H_{2'}$), 3.17 (s, 3 H, OCH_3), 4.15 (m, 1 H, $H_{1'}$), 4.32 (m, 2 H, $H_{5'}$), 4.54 (s, 2 H, OCH_2O), 6.20 (brs, 1 H, $H_{4'}$), 7.26 (s, 2 H, NH_2), 7.89 (s, 1 H, H_8) and 8.16 (s, 1 H, H_2); ^{13}C NMR Z-Isomer: 12.27 ($C_{2'}$), 25.89 ($C_{1'}$), 55.12 (OCH_3), 66.56 ($C_{5'}$), 95.62 (OCH_2O), 121.44 ($C_{3'}$), 123.19 ($C_{4'}$), 119.22 (C_5), 139.75 (C_8), 150.83 (C_4), 152.96 (C_2), 156.35 (C_6); *E*-Isomer: 12.04 ($C_{2'}$), 26.38 ($C_{1'}$), 55.12 (OCH_3), 66.88 ($C_{5'}$), 95.79 (OCH_2O), 121.05 ($C_{3'}$), 122.75 ($C_{4'}$), 119.39 (C_5), 139.75 (C8), 150.83 (C4), 152.96 (C_2), 156.35 (C_6).

(*Z*)- and (*E*)-9-[(2-Hydroxyethylidene)cyclopropyl]adenine (3) and (4). A solution of the protected *E/Z*-isomers **8** (1.35 g, 5.17 mmol) in methanolic HCl (0.3 M, 80 mL) was refluxed for 2.5 h. Volatile components were evaporated to give a sirup which was briefly stirred in methanolic ammonia (20 %, 30 mL) at 0°C. After evaporation, the residue was chromatographed on silica gel using CH_2Cl_2 - MeOH (9 : 1 → 4 : 1, containing 0.1 % NH_3). The *Z*-isomer **3** was eluted first (623 mg, 55.5 %), mp 223-225°C. UV_{max} (EtOH) 260 nm (ε 14 400); ^1H NMR δ 1.62 (d x qt, 1 H, ^2J = 10.5 Hz, $^3J_{trans} = {}^4J = {}^5J$ = 2.7 Hz, $H_{2''}$) and 1.86 (ddq, 1 H, ^2J = 10.0 Hz, $^3J_{cis}$ = 7.8 Hz, $^4J = {}^5J$ = 2.1 Hz, $H_{2'}$), 4.08 (ddd, 1 H, $^3J_{cis}$ = 7.1 Hz, $^3J_{trans}$ = 2.7 Hz, ^4J = 1.2 Hz, $H_{1'}$), 4.20 - 4.30 (brd, 2 H, apparent J = 2.4 Hz, $H_{5'}$), 5.39 (t, 1 H, ^3J = 5.6 Hz, OH), 6.20 (td, 1 H, ^3J = 5.1 Hz, ^4J = 2.1 Hz, $H_{4'}$), 7.25 (s, 2 H, NH_2), 7.98 (s, 1 H, H_8) and 8.14 (s, 1 H, H_2); ^{13}C NMR 11.46 ($C_{2'}$), 26.37 ($C_{1'}$), 61.26 ($C_{5'}$), 119.13 ($C_{3'}$), 125.31 ($C_{4'}$), 119.37 (C_5), 140.43 (C_8), 150.77 (C_4), 152.865 (C_2), 156.46 (C_6); HRMS, calcd for $C_{10}H_{11}N_5O$ (M) 217.0964. Found 217.0960. For

$C_{10}H_{11}N_5O$ calcd C, 55.29; H, 5.10; N, 32.24. Found: C, 55.47; H, 5.27; N, 32.44. Further elution gave the *E*-isomer **4** (385 mg, 34.3 %), mp 218-220°C. UV_{max} (EtOH) 260 nm (ε 14 400); ^1H NMR δ 1.68 (d x qt, 1 H, ^2J = 10.5 Hz, $^3J_{trans}$ = ^4J = ^5J = 2.7 Hz, $H_{2''}$) and 1.94 (ddq, 1 H, ^2J = 10.3 Hz, $^3J_{cis}$ = 7.7 Hz, ^4J = ^5J = 2.1 Hz, $H_{2'}$), 4.02 (ddd, 1 H, $^3J_{cis}$ = 7.2 Hz, $^3J_{trans}$ = 3.3 Hz, ^4J = 1.2 Hz, $H_{1'}$), 4.12 (td, 2 H, ^3J = 5.7 Hz, ^5J = 1.2 Hz, $H_{5'}$), 4.84 (t, 1 H, ^3J = 5.6 Hz, OH), 6.56 (tq, 1 H, ^3J = 5.1 Hz, ^4J = 1.2 Hz, $H_{4'}$), 7.21 (s, 2 H, NH_2), 8.01 (s, 1 H, H8) and 8.13 (s, 1 H, H_2); ^{13}C NMR 12.33 ($C_{2''}$), 25.49 ($C_{1''}$), 61.21 ($C_{5'}$), 120.15 ($C_{3'}$), 125.37 ($C_{4'}$), 119.26 (C_5), 140.38 (C_8), 150.01 (C_4), 153.05 (C_2), 156.42 (C_6); HRMS calcd for $C_{10}H_{10}N_5$ (M - H_2O + H) 200.0936. Found 200.0930. For $C_{10}H_{11}N_5O$ calcd C, 55.29; H, 5.10; N, 32.24. Found: C, 55.02; H, 5.12; N, 32.23.

Adenosine Deaminase Assay.[1] Compound **3** or **4** (0.56 mg, 2.6 μmol) was incubated with adenosine deaminase from calf intestine (0.36 units) in 0.05 M Na_2HPO_4 (pH 7.5, 0.4 mL) at room temperature. Aliquots were periodically withdrawn and examined by TLC (CH_2Cl_2 - MeOH, 9 : 1 + 1 drop of NH_4OH). The spots of starting compound and deamination product were eluted with ethanol and their concentration was determined by UV spectrophotometry. After 45 h, 82 % of the *E*-isomer **4** was deaminated whereas the *Z*-isomer **3** was essentially unchanged.

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