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The Enantiomers of Carbocyclic 5′-Norguanosine: Activity Towards Epstein–Barr Virus

Vasanthakumar Rajappan,[a] Stewart W. Schneller,[a,*] Stephanie L. Williams[b] and Earl R. Kern[b]

[a]*Department of Chemistry, Auburn University, Auburn, AL 36849-5312, USA*
[b]*Department of Pediatrics, Division of Clinical Virology, University of Alabama at Birmingham, Birmingham, AL 35294-2170, USA*

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Abstract—(−)-5′-Noraristeromycin (**1**) has shown antiviral activity towards, particularly cytomegalovirus, vaccinia virus and measles while its (+)-enantiomer (**2**) is effective towards hepatitis B virus. To determine if the antiviral characteristics of **1** and **2** extended to the guanine analogues (**3** and **4**), these enantiomers were prepared and evaluated against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), cytomegalovirus (CMV), varicella zoster virus (VZV), Epstein–Barr virus (EBV), human herpes virus type 6 (HHV-6), human herpes virus type 8 (HHV-8), vaccinia virus (VV), cowpox virus (CV), vesicular stomatitis virus (VSV), respiratory syncytial virus (RSV), hepatitis B virus (HBV), and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). The only activity found for **3** was for Epstein–Barr virus in VCA Elisa (EC_{50} 0.78 μg/mL), immunofluorescence assay for VCA or gp 350/250 (1.8–4.0 μg/mL) and DNA hybridization (EC_{50} 0.82 μg/mL) assays with no accompanying toxicity seen in the host Daudi cells. No activity was noted for **4**.

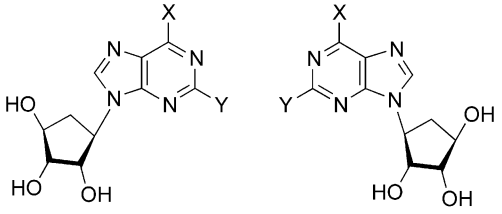
Introduction

For the last decade, we[1] have been investigating the antiviral properties of carbocyclic nucleosides[2] lacking the 5′-methylene unit (5′-nor carbanucleosides).[3] These efforts have proven fruitful with the two enantiomers of 5′-noraristeromycin (**1** and **2**).[4] To date, the enantiomers possessing the guanine base (**3** and **4**) have not been evaluated[5] but could be expected to have activity by correlation to the parent carba-guanosines[3] and analogues therefrom.[6] Compounds **3** and **4** are the subject of this report (Fig. 1).

Chemistry

Following the palladium promoted coupling of allylic acetates with purine bases pioneered so eloquently by Trost and his co-workers[7] reaction of (+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate (**5**)[8] with 2-amino-6-chloropurine (**6**) in the presence of *tetrakis*(triphenylphosphine)palladium gave a 42% yield of **7** (Scheme 1). Standard glycolization conditions (osmium tetroxide/4-methylmorpholine *N*-oxide) converted **7** into **8** (81%). Acidic hydrolysis of **8** produced the target compound **3**.

In view of the reports from Trost's laboratory[7] that it was likely the palladium coupling procedure (step *a*, Scheme 1) would also give the N-7 regioisomer of **7** and that the 'up' or β diastereomer would accompany the formation of **8** from **7**, it was necessary to confirm the structure of **8** as well as **3** as assigned. This was done by



1, X=NH_2; Y=H
3, X=OH; Y=NH_2

2, X=NH_2; Y=H
4, X=OH; Y=NH_2

Figure 1.

*Corresponding author. Tel.: +1-334-844-5737; fax: +1-334-844-5748; e-mail: schnest@auburn.edu

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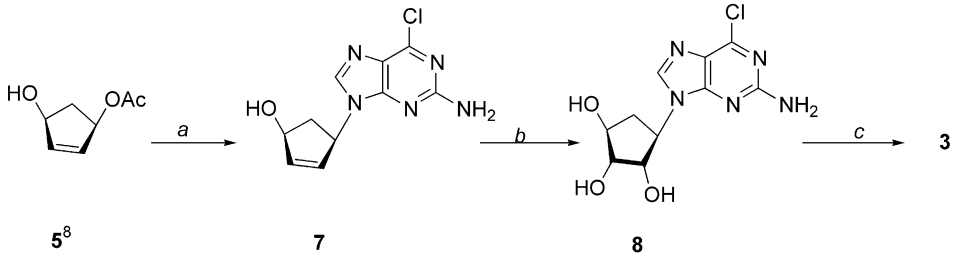

Scheme 1. Reaction conditions: (a), NaH, 2-amino-6-chloropurine (**6**), $(PPh_3)_4Pd/PPh_3$, THF, 60 °C, 72 h (42%); (b), OsO_4/*N*-methylmorpholine *N*-oxide, THF/H_2O, rt, 24 h (81%); (c), 1 N HCl, reflux, 5 h (71%).

the favorable comparison of the ^{13}C NMR analysis of these two compounds with their racemic counterparts that were prepared by a route that gave the N-9 regioisomer and the 'down' glycol structural components unambiguously.[5b]

By an analogous route, **4** was obtained from the enantiomer of **5**.[4b]

Antiviral Results

Compounds **3** and **4** were evaluated[4,9] against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), cytomegalovirus (CMV), varicella zoster virus (VZV), Epstein–Barr virus (EBV), human herpes virus 6 (HHV-6), human herpes virus 8 (HHV-8), vaccinia virus (VV), cowpox virus (CV), vesicular stomatitis virus (VSV), respiratory syncytial virus (RSV), hepatitis B virus (HBV), and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2).

From this analysis, **3** was found to have significant activity towards Epstein–Barr virus in three assays (see Table 1): VCA and gp 350/250 immunofluoroescent assay (1.8–4.0 μg/mL), VCA Elisa (EC_{50} 0.78 μg/mL), and DNA hybridization (EC_{50} 0.82 μg/mL) (acyclovir in these three assays: EC_{50} 1.3–2.7 μg/mL, EC_{50} 1.7 μg/mL, and EC_{50} 0.7 μg/mL, respectively). In these assays, there was no toxicity seen ($IC_{50} > 50$ μg/mL) towards the host (Daudi) cells. No other antiviral activity was found for **3** and **4**.

Conclusions

A convenient synthetic route, unencumbered by structural ambiguity, has been achieved for both enantiomers of carbocyclic 5′-norguanosine. The D-like analogue **3** has shown promising effects towards Epstein–Barr virus. Interestingly, another gamma herpes virus, HHV-8, was not inhibited by **3**. As a consequence, the compound is now serving as a structural prototype for new leads into carbocyclic nucleosides[3] as a source of new anti-EBV agents for which there is a clear need.[10]

Experimental

Chemistry

Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer (operated at 250 or 62.5 MHz, respectively). All ^1H chemical shifts are reported in δ relative to internal standard tetramethylsilane (TMS, δ 0.00). ^{13}C chemical shifts are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.23) or relative to DMSO-d_6 (center of septet, δ 39.51). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublet), dt (doublet of triplet) and m (multiplet). Optical rotations were determined using the sodium-D line on a JASCO DIP-360 polarimeter. Elemental analyses were performed by either M-H-W-Laboratories, Phoenix, AZ,

Table 1. Antiviral efficiency and toxicity against Epstein–Barr virus in Daudi cells

	IFA			ELISA			DNA		
	EC_{50}[a]	IC_{50}	CC_{50}	EC_{50}	IC_{50}	CC_{50}	EC_{50}	IC_{50}	CC_{50}
ACV	2.74±0.87[b]	>50	>50	1.66±0.38	>50	>50	0.66±0.31	>50	>50
	1.28±0.57[c]	>50	>50						
3	1.80±0.28[b]	>50	>50	0.78±0.19	>50	>50	0.82±0.43	>50	>50
	4.04±4.14[c]	>50	>50						
4	>50[b]	>50	>50	>50	>50	>50	>50	>50	>50
	>50[c]	>50	>50						

[a]All values are given in μ/mL and the mean/standard deviation for at least two assays.
[b]Stained with VCA gp-125.
[c]Stained with gp 350/250.

USA, or Atlantic Microlabs, Atlanta, GA, USA. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm E. Merck silica gel 60-F_{254} precoated silica gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Whatman silica gel (average particle size 5–25 μm, 60 Å) and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

2-Amino-6-chloro-9-[(1′*R*,4′*S*)-4′-hydroxycyclopent-2′-en-1′-yl]-purine (7). To a suspension of 95% NaH (120 mg, 4.75 mmol) in anhydrous DMSO (12 mL) was added 2-amino-6-chloropurine (**6**) (1 g, 4.75 mmol) and the mixture was stirred at room temperature for 30 min. *Tetrakis*(triphenyphosphine)palladium [$(PPh_3)_4Pd$] catalyst (554.6 mg, 0.48 mmol) and triphenylphosphine (Ph_3P)(188.8 mg, 0.72 mmol) were added to the reaction mixture followed by a solution of **5**[8] (674.5 mg, 4.75 mmol) in freshly distilled THF (15 mL). The reaction mixture was stirred in an oil bath at 60 °C for 3 days, until tlc analysis (6:1, CH_2Cl_2/MeOH) showed substantial product formation. The volatiles were removed under reduced pressure. The residue was slurried in CH_2Cl_2 (50 mL) and filtered through a pad of Celite and the filtrate was evaporated under reduced pressure on a rotary evaporator. The resultant oil was loaded on a silica gel column and the faster moving impurities were eluted first using hexane/EtOAc (10:1). The product was eluted with EtOAc. Evaporation of the solvent and drying of the residue over P_2O_5 in vacuum afforded 500 mg (42%) of compound **7** as a light-yellow powder. An analytical sample was obtained by recrystallization from EtOAc/ether mixture: mp 165 °C [lit.[5b] (±)-racemic **7** mp 156–158 °C]; ^{1}H NMR (DMSO-d_6) δ 8.04 (s, 1H, CH), 6.93 (s, 2H, NH_2), 6.20 (dd, J = 2.5, 5 Hz, 1H, CH), 6.01 (dd, J = 2.5, 5 Hz, 1H, CH), 5.31 (dt, J = 2.5, 5 Hz, 1H, CH), 4.71 (d, J = 5 Hz, 1H, OH), 4.11 (m, 1H, CH), 2.86 (m, 1H, CH), 1.67 (m, 1H, CH); ^{13}C NMR (DMSO-d_6) δ 159.73, 153.57, 149.41, 141.27, 139.76, 130.52, 123.54, 73.64, 56.89, 41.21. Anal. calcd for $C_{10}H_{10}N_5ClO$: C, 47.72; H, 4.00; N, 27.83; Cl, 14.09. Found: C, 47.61; H, 4.09; N, 27.88; Cl, 14.17.

Following an analoguous procedure, 2-amino-6-chloro-9-[(1′*S*,4′*R*)-4′-hydroxycyclopent-2′-en-1′-yl]purine (ent-7)[11] was obtained using the enantiomer of **5**.[4b] Anal. calcd for $C_{10}H_{10}N_5ClO$: C, 47.72; H, 4.00; N, 27.83; Cl, 14.09. Found: C, 47.55; H, 4.06; N, 27.98; Cl, 14.16.

2-Amino-6-chloro-9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-trihydroxycyclopentyl]purine (8). To a solution of **7** (720 mg, 3.01 mmol) and 50% aqueous solution of 4-methylmorpholine *N*-oxide (2 mL, 10 mmol) in a mixture of THF (20 mL) and H_2O (enough to make the solution clear) was added osmium tetroxide (OsO_4) (75 mg) in one portion. After stirring this solution at room temperature for 24 h, sodium bisulfite (300 mg) was added to the reaction and allowed to stir for another 30 min. The solvents were removed under reduced pressure. The thick brown residue was mixed with silica gel (5 g) in CH_2Cl_2/MeOH (4:1) to make a slurry and it was loaded on a silica gel column. The column was first eluted with CH_2Cl_2/MeOH (20:1) to remove the faster moving impurities and then with CH_2Cl_2/MeOH (3:1) to obtain the desired compound. After evaporation of the solvent 700 mg (81%) of compound **8** was obtained as an off white powder. Recrystallization from a mixture of EtOAc/MeOH/hexane afforded an analytically pure sample: mp 210–211 °C. ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H, CH), 6.90 (s, 2H, NH_2), 5.19 (d, J = 5 Hz, 1H, OH), 5.08 (d, J = 7.5 Hz, 1H, OH), 4.88 (d, J = 5 Hz, 1H, OH), 4.62 (dt, J = 10, 7.5 Hz, 1H, CH), 4.49 (m, 1H, CH), 3.90 (d, J = 2.5 Hz, 1H, CH), 3.76 (s, 1H, CH), 2.57 (m, 1H, CH), 1.70 (m, 1H, CH); ^{13}C NMR (DMSO-d_6) δ 159.58, 154.40, 151.09, 149.36, 141.85, 76.58, 75.19, 73.55, 57.88, 36.60. Anal. calcd for $C_{10}H_{12}ClN_5O_3$: C, 42.04; H, 4.23; N, 24.51; Cl, 12.41. Found: C, 42.29; H, 4.32; N, 24.23; Cl, 12.55.

2-Amino-6-chloro-9-[(1′*S*,2′*R*,3′*S*,4′*R*)-2′,3′,4′-trihydroxycyclo-pentyl]purine (ent-8). This was obtained following the same procedure as just described for **8** beginning with ent-**7**. Anal. calcd for $C_{10}H_{12}ClN_5O_3 \cdot 0.1H_2O$: C, 41.78; H, 4.28; N, 24.36; Cl, 12.33. Found: C, 41.48; H, 4.34; N, 24.05; Cl, 12.09.

1-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentyl]guanine (3). Compound **8** (120 mg, 0.42 mmol) was dissolved in 1 N aq HCl (20 mL) and this solution was refluxed for 5 h under an atmosphere of N_2. The solvent was removed by rotary evaporation and the residue was azeotroped with EtOH (2×20 mL). The resultant yellow residue was dissolved in distilled H_2O (10 mL) and the pH of the solution was adjusted to 7 using 3 N NaOH. The turbid mixture was refrigerated overnight and the precipitate that formed was recovered by filtration and dried over P_2O_5 under vacuum to provide 80 mg (71%) of **5** as an off white powder. Recrystallization of this material from H_2O/MeOH gave an analytical sample of **3**: mp > 280 °C [lit.[5b] for (±)-racemic **3** mp > 282 °C]; $[\alpha]_D^{25}$ −15.8° (*c* 0.43, DMSO); ^{1}H NMR (DMSO-d_6) δ 10.56 (s, 1H, NH), 7.76 (s, 1H, CH), 6.41 (s, 2H, NH_2), 5.17 (d, J = 2.5 Hz, 1H, OH), 5.01 (d, J = 5 Hz, 1H, OH), 4.83 (d, J = 5 Hz, 1H, OH), 4.58 (dt, J = 7.5, 7.5 Hz, 1H, CH), 4.43 (m, 1H, CH), 3.87 (d, J = 5 Hz, 1H, CH), 3.74 (m, 1H, CH), 2.60 (m, 1H, CH), 1.66 (m, 1H, CH); ^{13}C NMR (DMSO-d_6) δ 157.09, 153.39, 151.49, 136.15, 116.73, 76.76, 75.52, 73.64, 57.51, 37.17. Anal. calcd for $C_{10}H_{13}N_5O \cdot 1.5\ H_2O$: C, 40.82; H, 5.48; N, 23.80. Found: C, 41.20; H, 5.41; N, 23.90.

Employing the same procedure, 1-[(1′*S*,2′*R*,3′*S*,4′*R*)-2′,3′,4′-trihydroxycyclopentyl]guanine (ent-**3**) was obtained in the same way from ent-**8**; $[\alpha]_D^{25}$ +10.4° (*c* 0.46, DMSO). Anal. calcd for $C_{10}H_{13}N_5O \cdot 0.5H_2O$: C, 43.48; H, 5.11; N, 25.35. Found: C, 43.19; H, 5.17; N, 25.17.

Antiviral assays

The antiviral and toxicity analyses were performed following standard procedures in the literature: for HSV, CMV, VZV, VV and cowpox;[9] for VSV, RSV, and

HIV;[4a] for HBV.[4b] The assays for EBV activity were conducted as follows: Daudi cells were superinfected with P3HR-1 virus and allowed to incubate at 37 °C for approximately 1 h. After incubation, the infected cells were washed and the appropriate concentrations of drug added to the cells. The cells were then incubated for 3 days, after which they were utilized for immunofluorescence (IFA), ELISA assays or DNA hybridization.

Immunofluorescent assay for EBV activity

Slides were prepared by spotting 25 μL of infected cell on slides. The slides were allowed to air dry and fixed in acetone. After fixation, the slides were washed three times with PBS, air dried and 25 μL of either mouse anti-EBV VCA gp-125 or Ma-gp 350/250 was added to individual spots. After 37 °C incubation for 1 h, slides were washed with PBS and FITC AffiniPure Goat Anti-Mouse IgG + M was added to the spots. After incubation, 500 cells per spot were counted using a Nikon fluroescence microscope and the percentage of cells positive for VCA was calculated. EC_{50} values were determined using a computer program (MacSynergy II).

ELISA assay for EBV activity

Cells were added to triplicate wells in 96-well plates at the appropriate concentration and allowed to air dry. The cells were then fixed with an AcOH/EtOH solution for 20 min at room temperature. Plates were then rinsed three times with PBS, mouse anti-EBV VCA gp-125 added to the wells and incubated at 37 °C for 1 h. Plates were rinsed with horseradish peroxidase; labeled goat anti-mouse IgG_1 was added to the wells and incubated at 37 °C for 30 min. Plates were rinsed with PBS and *o*-phenylenediamine in a citrate buffer with H_2O_2 added to the plates. The plates were covered with foil and shaken for approximately 10 min at room temperature. The reaction was stopped with 3 N H_2SO_4 and wells read on a microplate reader at 492 nm. EC_{50} values were calculated as described above.

DNA in situ hybridization assay for EBV activity

Slides were prepared as described above and a Simply Sensitive Horseradish Peroxidase-AEC In situ Detection System was used to determine the effect of the antiviral compounds on EBV DNA synthesis. The staining and detection were performed to the manufacturer's instructions. Slides were viewed using a light microscope, 500 cells/spot were counted, the percentage of positive cells calculated and EC_{50} values determined.

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