Notes

Synthesis and Biological Evaluation of Acyclic Neplanocin Analogues

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Acyclic neplanocin analogues were prepared by condensation of adenine or N^2-acetylguanine with (*E*)-1,4-dichlorobut-2-ene and subsequent hydrolysis. The N-9-substituted product 9-[(*E*)-4-hydroxybut-2-enyl]adenine (**5**) was obtained when adenine was employed as the starting purine, while N^2-acetylguanine yielded both the N-7 and N-9 isomers. Cell-culture studies revealed that only the chloro-substituted intermediate 9-[(*E*)-4-chlorobut-2-enyl]adenine (**4**) exhibited significant cytotoxicity against P-388 mouse lymphoid leukemia cells, while the N-9-substituted guanine analogue 9-[(*E*)-4-hydroxybut-2-enyl]guanine (**10**) inhibited replication of herpes simplex viruses type 1 and type 2.

Initial reports of the antiviral activity of acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine (**1**)[1,2] (Figure 1), have stimulated the preparation and antiviral evaluation of other acyclic nucleosides. In fact, the design of acyclovir itself was based on earlier studies,[3] which had shown that the intact cyclic carbohydrate moiety was not always necessary for nucleosides to act as substrates for enzymes. Thus, the early study demonstrated that acycloadenosine (**2**) was an efficient substrate for adenosine deaminase, presumably due to its ability to assume a conformation that is superimposable with the conformation of adenosine with respect to the adenine group, C-1′, ether oxygen, C-4′, C-5′, and the 5′-OH.[2]

More recently, a novel class of carbocyclic nucleosides has been described in which the ribose moiety is replaced by a cyclopentene ring.[3-5] Neplanocins A, B, and C have antitumor activities in mice, with neplanocin A (Figure 1) being more potent than many of the currently used antitumor drugs.[6] Neplanocin A was also found to inhibit vaccinia virus replication in vitro.[7] These observations, coupled with the earlier discoveries that acyclonucleosides can sometimes mimic the corresponding nucleosides possessing the entire sugar moiety, provided an excellent rationale for the synthesis of acyclic neplanocin analogues. Also, since previous studies[1,8] with other antitumor and antiviral nucleosides suggested that purine derivatives other than adenine should be explored in cases where an adenine nucleoside exhibits antitumor or antiviral properties, we have prepared both the adenine and guanine acyclic neplanocin analogues.

Table I. Ultraviolet Spectral Data and R_f Values of Alkylated Guanines

	λ_{max}, nm			
compd	0.1 N HCl	H_2O	0.1 N NaOH	R_f
7	263	261	264	0.47[a]
8	263	265	269	0.60[a]
9	263	259	264	0.27[a]
10	253	252	268	0.50[b]
11	262	265	268	0.38[a]
12	250	283	280	0.59[b]

[a] Solvent used was $CHCl_3$–MeOH (3:1). [b] Solvent used was *i*-PrOH–NH_4OH–H_2O (7:1:2).

Chemistry. The synthesis of acycloneplanocin (**5**) is outlined in Scheme I. Adenine was condensed with (*E*)-1,4-dichlorobut-2-ene in the presence of sodium hydride. The corresponding 9-substituted adenine (**4**), obtained in 47% yield, was treated with fresh cuprous chloride solution in the presence of hydrochloric acid. Pure **5** was isolated in 81% yield after purification by flash chromatography on a silica gel column. For the preparation of the guanine derivative, N^2-acetylguanine (**6**) was condensed with *trans*-(*E*)-1,4-dichlorobut-2-ene in anhydrous DMF in the presence of triethylamine (Scheme II). The N-9 (**7**) and N-7 (**8**) isomers were obtained in a 1:3.5 ratio. The isomers were easily separated and converted to the corresponding acyclic nucleosides **10** and **12** by cuprous chloride/hydrochloric acid hydrolysis and subsequent deprotection of **9** and **11** using methylamine.

The assignment of the N-9 and N-7 isomers of the substituted guanines were based on the spectral properties.[9] The λ_{max} values are presented in Table I, and the assignments are consistent with observed spectra for other 9- and 7-substituted guanines.[9a-c] The ^1H NMR properties are summarized in Table II. The 9- and 7-alkylated guanines can be firmly identified on the basis of previous observations that the chemical shift for the H-8 proton of the N-7 isomer is always downfield from that of the N-9 isomer.[9]

(1) (a) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716. (b) Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature, (London)* **1978**, *272*, 583.
(2) Schaeffer, H. J.; Gurwara, S.; Vince, R.; Bittner, S. J. *J. Med. Chem.* **1971**, *14*, 367.
(3) Hayashi, M.; Yaginuma, S.; Muto, N.; Tsujino, M. *Nucleic Acids Symp. Ser.* **1980**, *8*, 565.
(4) Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nokatsu, K. *J. Antibiot.* **1981**, *34*, 675.
(5) Yaginuma, S.; Muto, N.; Tsujino, N.; Sudate, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 359.
(6) Tsujino, M.; Yaginuma, S.; Fujii, T.; Hayano, K.; Matsuda, T.; Watanabe, T.; Abe, J. In *Current Chemotherapy and Infectious Disease*; Nelso, J., Gassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; Vol. 2, pp 1559–1561.
(7) Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. *J. Biol. Chem.* **1984**, *259*, 4353.
(8) Lee, H. J.; Vince, R. *J. Pharm. Sci.* **1980**, *69*, 1019.
(9) (a) Ogilvie, K. K.; Proba, Z. A. *Nucleosides Nucleotides* **1984**, *3*, 537. (b) Ogilvie, K. K.; Cheriyan, U. O.; Radatus, B. K. *Can. J. Chem.* **1982**, *60*, 3005. (c) Liu, M. C.; Kumich, S.; Lin, T. S. *Tetrahedron Lett.* **1984**, *25*, 613. (d) Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759.

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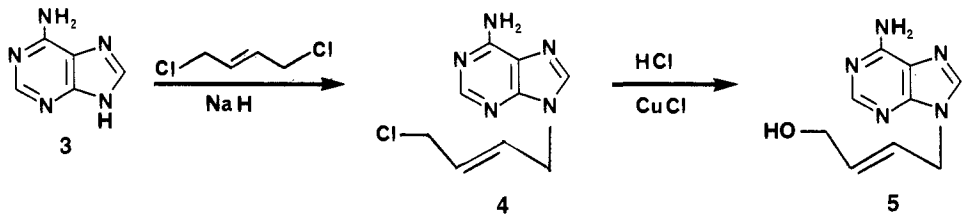
Scheme I



Scheme II

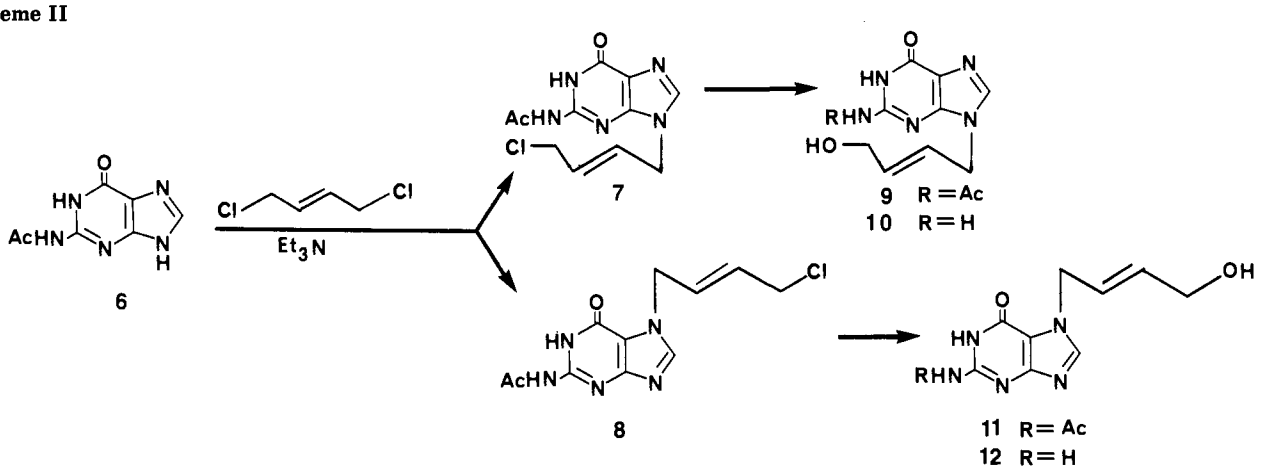


Table II. Proton Magnetic Resonance Data (δ)

compd	CH_3CO	H-4′	H-1′	OH	H-2′ and H-3′	NH_2	H-8	N^2H	H-1
7	2.17	4.15	4.70		5.52–6.09		7.94	11.72	12.02
8	2.17	4.15	4.90		5.62–6.12		8.15	11.50	12.11
9	2.18	3.90	4.66	4.74	5.52–5.90		7.95	11.71	12.01
10		3.89	4.55	4.74	5.50–5.83	6.43	7.69		10.55
11	2.17	3.89	4.90	4.79	5.62–5.90		8.15	11.56	12.07
12		3.90	4.70	4.80	5.52–5.91	6.13	7.88		10.77

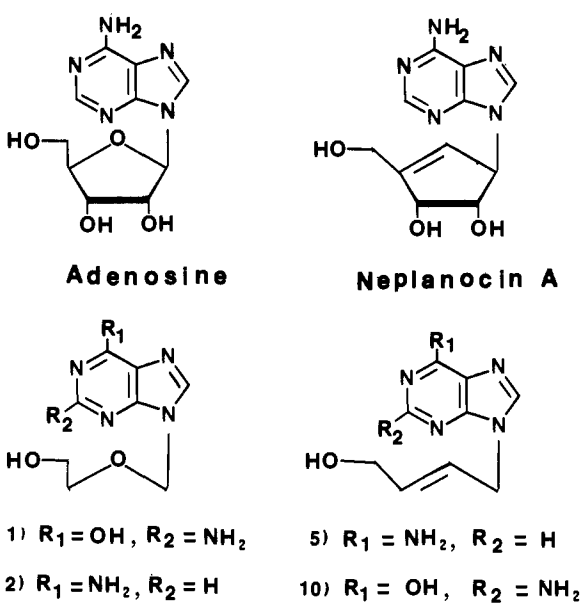


Figure 1.

Results

Cytotoxicities of the acyclic neplanocin analogues were evaluated with use of P388 mouse lymphoid leukemia cells as described in the Experimental Section. Only the chloro derivative **4** exhibited significant growth inhibition in this assay (ED_{50} = 5 ± 0.1 μg/mL). Each analogue was examined for in vitro antiviral activity against herpes simplex viruses type 1 (377) and type 2 (HF) by the quantitative determination of its ability to inhibit virus-induced cytopathogenic effects (CPE) in infected cultures with use of pregrown Vero cell monolayer cultures as the host cell system. Only the guanine analogue **10** exhibited significant activity. A virus rating (VR) was calculated for the activity of **10** against each virus where VR = a measurment of selective antiviral activity that takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al.[10] Thus, **10** exhibited VR's of 1.5 (ED_{50} = 172 μg/mL) and 0.9 (ED_{50} = 219 μg/mL) against HSV-1 and HSV-2, respectively.[11] The VR's of acyclovir were 6.3 (ED_{50} = 12.3 μg/mL) for HSV-1 and 4.8 (ED_{50} = 4.8 μg/mL) for HSV-2. Thus, it appears that the acyclic neplanocin analogues offer no significant advantages over the parent compounds.

Experimental Section

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra were obtained on JEOL FX 90QFT or Nicollet NT300 spectrometers and were recorded in Me_2SO-d_6. Chemical shifts are expressed in ppm downfield from Me_4Si. IR spectra were determined with KBr pellets on a Perkin-Elmer 281 spectrometer, and UV spectra were determined on a Beckman DU-8 spectro-

(10) Ehrlich, J.; Sloan, B. J.; Miller, F. A.; Machamer, H. *Ann. N.Y. Acad. Sci.* **1965**, *130*, 5.

(11) A VR > 1.0 indicates definite (+) antiviral activity, a VR of 0.5–0.9 indicates marginal to moderate (±) activity, and a VR < 0.5 usually indicates no (−) significant antiviral activity. Ara-A used as a positive control exhibited VR's of 2.0 and 1.4 against HSV-1 and HSV-2, respectively.

photometer. Mass spectra were obtained with an AEI Scientific Apparatus Limited MS-30 mass spectrometer. Thin-layer chromatography (TLC) was performed on 0.25-mm layers of Merck silica gel 60F-254 and column chromatography on Merck 60 (230–400 mesh). All chemicals and solvents are reagent grade unless otherwise specified. Anhydrous DMF and N^2-acetylguanine were obtained from Aldrich Chemical Co. Triethylamine was distilled over sodium and benzophenone. All reactions were carried out under nitrogen.

9-[(*E*)-4-Chlorobut-2-enyl]adenine (4). To a stirred solution of 1.0 g (7.4 mmol) of adenine in 100 mL of anhydrous DMF was added 0.40 g (50% in oil, 8.3 mmol) of sodium hydride. After evolution of hydrogen had ceased, the mixture was cooled to −40 °C and 25 mL (230 mmol) of (*E*)-1,4-dichlorobut-2-ene was dropped into the slurry. The stirred mixture was allowed to warm slowly to room temperature over a period of 3 h and kept at room temperature for 1 h. The insoluble salt was removed by filtration, and the volatile material was removed in vacuo. The residue was triturated with ethyl ether (3 × 15 mL). A pale yellow solid product was obtained (1.7 g) and recrystallized from 7 mL of methanol to yield white crystals, 780 mg (47%): mp 246 °C dec; TLC R_f 0.50 ($CHCl_3$–MeOH, 3:1); IR 3400–3100 (NH_2), 1680, 1610, 1600 (C=C, C=N) cm^{-1}; ^1H NMR δ 4.17 (d, 2 H, H-4′), 4.81 (d, 2 H, H-1′), 5.77–6.06 (2 d, t, 2 H, vinyl H-2′ and H-3′, J = 15 Hz), 7.28 (s, 2 H, NH_2), 8.14 and 8.16 (2 s, 2 H, H-8 and H-2). Anal. ($C_9H_{10}N_5Cl$) C, H, N.

9-[(*E*)-4-Hydroxybut-2-enyl]adenine (5). To a solution of 447 mg (2.0 mmol) of 4 in 1.5% hydrochloric acid (80 mL) was added 10 mL of fresh cuprous chloride solution (3.5 mg of CuCl/mL). The mixture was warmed at 40 °C for 3 h, neutralized to pH 7 with 1 N sodium hydroxide, and concentrated to dryness. The residue was extracted with methanol, and the methanol solution was absorbed onto 1.2 g of silica gel. The sample was applied to a flash column (1.8 × 16 cm) and eluted with chloroform–methanol (4:1). The product fractions were combined and concentrated to obtain 334 mg (81%) as white crystals: mp 200–202 °C; TLC R_f 0.29 ($CHCl_3$–MeOH, 3:1); IR 3400–3000 (NH_2, OH), 1690, 1610 (C=C, C=N) cm^{-1}; ^1H NMR δ 3.80 (s, 1 H, OH), 3.85 (d, 2 H, H-4′), 4.80 (d, 2 H, H-1′), 5.66–5.79 (2 d, t, 2 H, vinyl H-2′ and H-3′, J = 15 Hz), 7.18 (s, 2 H, NH_2), 8.10 and 8.14 (2 s, 2 H, H-8 and H-2). Anal. ($C_9H_{11}N_{50}$) C, H, N.

N^2-Acetyl-9-[(*E*)-4-chlorobut-2-enyl]guanine (7) and N^2-Acetyl-7-[(*E*)-4-chlorobut-2-enyl]guanine (8). To a suspension of 1.93 g (10 mmol) of N^2-acetylguanine in 100 mL of anhydrous DMF were added 2.1 mL (15 mmol) of anhydrous triethylamine and 18 mL (170 mmol) of (*E*)-1,4-dichlorobut-2-ene. The reaction mixture was stirred at room temperature for 24 h and at 50–60 °C for 10 h. The volatile material was evaporated in vacuo, and the residual syrup was triturated with petroleum ether and ethyl ether. The residue was dissolved in methanol and absorbed onto 8.0 g of silica gel applied to a flash column (4 × 14.5 cm) and eluted with chloroform–methanol (40:1, 500 mL; 30:1, 200 mL; 20:1, 300 mL). The low-R_f fraction was concentrated and gave the 9-substituted isomer **7**, yield 240 mg (8.5%): mp 252 °C dec after recrystallization from methanol–chloroform–ether (1:1:3); IR 3400–3050 (NH), 1690, 1670, 1605 (C=O, C=C, C=N) cm^{-1}; MS (20 eV, 200 °C), m/e 254 (M^+ − 27), 193 (AcB^+), 151 (B^+), 109, 60, 43; UV, NMR, and TLC data are collected in Tables I and II. Anal. ($C_{11}H_{12}ClN_5O_2$) C, H, N.

The high-R_f fraction was concentrated and gave the pure 7-substituted isomer (**8**), yield 826 mg (29.4%): mp 254 °C dec; IR 3400–3050 (NH), 1750, 1710, 1690, 1630 (C=O, C=C, C=N) cm^{-1}; MS (20 eV, 200 °C), m/e 281 (M^+), 193 (AcB^+), 151 (B^+), 59, 41; UV, NMR, and TLC data are collected in Tables I and II. Anal. ($C_{11}H_{12}ClN_5O_2$) C, H, N.

N^2-Acetyl-9-[(*E*)-4-hydroxybut-2-enyl]guanine (9). To a solution of 123 mg (0.49 mmol) of **7** in 20 mL of 1.5% hydrochloric acid was added 2.5 mL of fresh cuprous chloride solution (3.5 mg of CuCl/mL). The mixture was warmed at 40 °C for 1.5 h, neutralized to pH 7 with 1 N sodium hydroxide, and then concentrated and absorbed onto 0.8 g of silica gel. The absorbed sample was applied to a flash column (1.8 × 14 cm) and eluted with chloroform–methanol (5:1, 300 mL). The product fractions (7–19, 10 mL each) were combined and concentrated to obtain **9** as a pure white solid, yield 41 mg (36%), mp 218–220 °C. It can be recrystallized from ethanol: mp 218–220 °C; IR 3400–3000 (NH, OH), 1720, 1710, 1680, 1620 (C=O, C=N, C=C) cm^{-1}; MS (20 eV, 200 °C), m/e 263 (M^+), 244 (M_+ − 19), 190 (AcB^+ − 3), 151 (B^+), 107, 91, 60, 43; UV, NMR, and TLC data are collected in Tables I and II. Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

9-[(*E*)-4-Hydroxybut-2-enyl]guanine (10). Two hundred milligrams (0.76 mmol) of **9** was dissolved in 10 mL of 40% methylamine solution. The mixture was refluxed for 1.5 h under nitrogen, and the solvent was evaporated and coevaporated with methanol. The crude product was recrystallized from ethanol–water (1:1), yield 113 mg (67%), mp 270–272 °C. Further purification from ethanol–water (1:2) gave the following: mp 274–276 °C; IR 3500–3100 (NH, OH), 1700, 1675, 1630, 1610 (C=O, C=C, C=N) cm^{-1}; MS (70 eV, 200 °C), m/e 221 (M^+), 202 (M^+ − 19), 190 (AcB^+ − 3), 151 (B^+), 109, 67, 44; UV, NMR, and TLC data are collected in Tables I and II. Anal. ($C_9H_{11}N_5O_2 \cdot H_2O$) C, H, N.

N^2-Acetyl-7-[(*E*)-4-hydroxybut-2-enyl]guanine (11). To a solution of 1.30 g (4.6 mmol) of **8** in 160 mL of 1.5% hydrochloric acid was added 50 mL of fresh cuprous chloride solution (3.5 mg of CuCl/mL). The mixture was warmed at 42–45 °C for 12 h, neutralized, concentrated, and absorbed onto 2 g of silica gel. The absorbed sample was applied to a flash column (4.0 × 15 cm) and eluted with chloroform–methanol (5:1). The product fractions were combined and concentrated to obtain **11** as a white solid, yield 284 mg (23%), mp 219–221 °C. Recrystallization from ethanol gave the pure compound: mp 228–230 °C; IR 3500–3050 (NH, OH), 1695, 1680, 1615 (C=O, C=C, C=N) cm^{-1}; MS (20 eV, 200 °C), m/e 263 (M^+), 244 (M^+ − 19), 194 (AcB^+ + 1), 151 (B^+), 107, 91, 43; UV, NMR, and TLC data are collected in Tables I and II. Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

7-[(*E*)-4-Hydroxybut-2-enyl]guanine (12). Compound **11** (188 mg, 0.7 mmol) was dissolved in 12 mL of 40% methylamine solution and refluxed for 1.5 h under nitrogen. The solvent was evaporated and coevaporated with methanol. The crude product was recrystallized from water, yield 119 mg (75%): mp 295 °C dec; IR 3500–3000 (NH, OH), 1690, 1670, 1620 (C=O, C=C, C=N) cm^{-1}; MS (20 eV, 200 °C), m/e 221 (M^+), 202 (M^+ − 19), 191 (AcB^+ − 2), 151 (B^+), 107, 91, 79, 44; UV, NMR, and TLC data are collected in Tables I and II. Anal. ($C_9H_{11}N_5O_2$) C, H, N.

P-388 in Vitro Assay. Twofold dilutions of neplanocin analogue were tested in duplicate sets of tubes inoculated with 4×10^5 P-388 cells in 4 mL of Fischer's medium with 10% horse serum. The tubes were plugged with silicone stoppers and incubated for 72 h at 37 °C at a 30° angle without agitation. Tubes were rotated twice daily. Cell growth was determined by cell count with a hemocytometer, and percent inhibition was calculated from the corresponding controls, correcting all counts for inoculum.

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Registry No. **3**, 73-24-5; **4**, 104715-56-2; **5**, 104715-57-3; **6**, 19962-37-9; **7**, 104715-58-4; **8**, 104715-59-5; **9**, 104715-60-8; **10**, 104715-61-9; **11**, 104715-62-0; **12**, 104715-63-1; (*E*)-1,4-dichlorobut-2-ene, 110-57-6.