Table II. Reaction Conditions and Characterization of Malonamide Derivatives

no.	reaction conditions: time, h	reaction conditions: temp, °C	postreaction diluent	% yield	recrystn solvent	mp, °C	(lit. mp or C, H, N det)
5	5.0	160	Et_2O	35	EtOAc	191-192	(lit.[20] 193)
6	5.0	163	EtOH	65		228-229	(lit.[17,18] 223; 225)
7	2.5	185	Et_2O	25	EtOH/acetone (5:1)	211-212	($C_{19}H_{20}N_4O_6$)
8	4.0	185	6 N HCl	31	nitrobenzene	239-242	(lit.[16] 243-245)
9	3.0	165-180	EtOAc	53	CH_3CN/MeOH/acetone (3:1:1)	228-230	($C_{19}H_{20}Cl_2N_2O_2$)
10	4.0	160-175	EtOH	38		256-257	(lit.[15] 261)[a]
11	6.5	168-170	EtOH wash	11		191-193	($C_{21}H_{26}N_2O_2$)
12	3.5	155-160	EtOH wash	60		255-257	(lit.[18,19] 250; 248)[a]
14	2.0	190	hot EtOH	34		232-234	(lit.[14] 234)
16	3.5	185	none	19	hot 0.15 M Na_2CO_3	247-248	(lit.[14] 235)[a]
17	3.0	160	acetone	(3)	CH_3CN (yield after recrystn)	193-195	($C_{21}H_{26}N_2O_4$)
18	3.0	160	hot 1.5 M Na_2CO_3	27		206-208	($C_{15}h_{14}N_2O_4 \cdot {}^1/_2H_2O$)
19[b]	6.0	49	cold 3 N HCl	71	hot EtOH wash	280-282	(lit.[23] >250)
20	6.0	210	2 N HCl	22		265-268	(lit.[23] >250)

[a] Due to variance from reported melting point, confirmatory elemental analyses (C, H, N) were obtained for these compounds. [b] Succinyl chloride in pyridine rather than diester condensation was employed (see ref 23).

removing the associated fat, muscle, and connective tissues. The glands were then cut up into small pieces and added to 120 mL of potassium phosphate buffer (100 mM, pH 8.0) containing 5 mM diethyl dithiocarbamate and 5 mM disodium EDTA. This mixture was then homogenized in a Waring blender for 2 min and centrifuged for 10 min at 12000*g*. The supernatant was filtered through several layers of cheesecloth to remove the fat and recentrifuged for 1.25 h at 100000*g*. The resulting supernatant was decanted, and the pelleted microsomes were cooled to −80 °C and lyophilized. The microsomes were obtained in yields of between 1.0 and 1.5 g, starting from 120 g of crude SSV. The microsomes were dissolved in EDTA buffer[26] (100 mM, pH 8.0) containing 1.0 mM phenol, 2.0 mM glutathione, and 1.5% v/v Tween-20 at a 1.0 mg/mL concentration for enzyme-inhibition studies. The specific activity of these SSV microsomes was 52 ± 4 nmol of O_2 min^{-1} (mg of microsomes)$^{-1}$.

2. Enzyme Assays. Enzyme activity was determined by measuring oxygen consumption in solution with a Yellow Springs Instrument Co. polarographic electrode (Clark oxygen electrode, YSI-5331) in conjunction with a Gilson oxygraph (Model K-1C) as previously described.[27] The YSI reaction chamber was modified to permit smaller sample volumes by uniformly rounding the bottom. The temperature of the reaction chamber was maintained at 37 ± 1 °C with a Haake FG water circulator. The enzyme reaction was initiated by the addition of sodium arachidonate solution (5 mg/mL) to provide a 100 μM final concentration (K_m = 5.9 μM)[28] in the 2-mL reaction chamber. All inhibitors were added as 100 mM solutions in Me_2SO for routine enzyme assays. The levels of Me_2SO used in the enzyme-inhibitor experiments had no effect on enzyme activity itself. Initial enzyme velocities (dO_2/dt) were obtained by measuring the slopes of the resulting oxygen concentration vs. time curves, and reported as a percent of uninhibited control. Values presented in Table I are the result of triplicate determinations expressed as the mean ± standard deviation.

(26) Wallach, D. P.; Daniels, E. G. *Biochim. Biophys. Acta* **1971**, *231*, 445-457.
(27) Rome, L. H.; Lands, W. E. M. *Prostaglandins* **1975**, *10*, 813-824.
(28) Corey, E. J.; Shih, C.; Cashman, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3581.

Synthesis and Biological Properties of 9-(*trans*-4-Hydroxy-2-buten-1-yl)adenine and Guanine: Open-Chain Analogues of Neplanocin A

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Alkylation of adenine (**5a**) or 2-amino-6-chloropurine (**5b**) with excess *trans*-1,4-dichloro-2-butene (4), effected by K_2CO_3 in dimethyl sulfoxide or tetra-*n*-butylammonium fluoride in tetrahydrofuran, led in 90-95% regioselectivity to 9-alkylpurines **6a** and **6b**. The title compounds **2a** and **2b** were obtained by refluxing intermediates **6a** and **6b** in 0.1 M NaOH or HCl. Adenine derivative **2a** is a substrate for adenosine deaminase whereas both **2a** and **2b** exhibit 50% inhibition of the growth of murine leukemia L 1210 cell culture at 1 mM concentration.

Open-chain nucleoside analogues lacking the 2′- or both 2′- and 3′-carbon fragments of ribofuranose moiety are the subject of considerable current interest. Thus, antiviral agent[1] acyclovir (**1a**) is derived from guanosine by removing the 2′,3′-carbon fragment. The corresponding adenine analogue **1b** has a much lower antiviral activity,[1] but it is a substrate for adenosine deaminase.[2] Biologically active open-chain analogues can also be derived from other nucleosides, e.g., 5-benzyluridine[3] (**1c**), as well as from some structurally related antibiotics. For example, N^9-(4-hydroxybutyl)adenine (**1d**), a weak inhibitor of adenosine deaminase,[4] can be regarded as an open-chain analogue of aristeromycin,[5] a naturally occurring carbocyclic analogue

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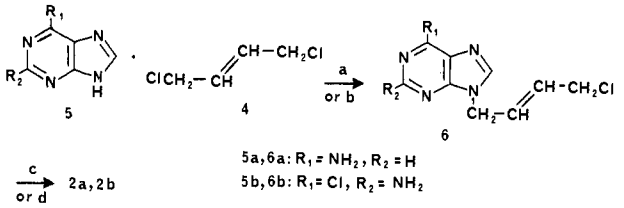
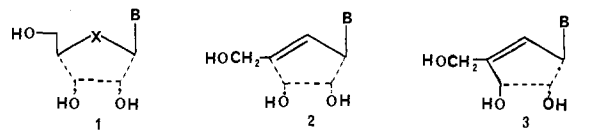
(1) Keller, P. M.; Fyfe, J. A.; Beauchamp, L.; Lubbers, C. M.; Furman, P.; Schaeffer, H. J.; Elion, G. B. *Biochem. Pharmacol.* **1981**, *30*, 3071.
(2) Schaeffer, H. J.; Gurwara, S.; Vince, R.; Bittner, S. *J. Med. Chem.* **1971**, *14*, 367.
(3) Niedzwicki, J. G.; el Kouni, M. H.; Chu, S. H.; Cha, S. *Biochem. Pharmacol.* **1981**, *30*, 2097.
(4) Schaeffer, H. J.; Vogel, D. *J. Med. Chem.* **1965**, *8*, 507.

0022-2623/87/1830-0437$01.50/0

Scheme I



of adenosine. The corresponding guanine derivative 1e exhibits antiviral activity,[1,6] albeit lower than acyclovir (1a).



1a: X = O, B = Gua
1b: X = O, B = Ade
1c: X = O, B = 5-C6H5CH2Ura
1d: X = CH2, B = Ade
1e: X = CH2, B = Gua

2a: B = Ade
2b: B = Gua

3a: B = Ade
3b: B = Gua

Recently, a new group of carbocyclic analogues of adenosine, neplanocins A–F, were isolated from a culture of *Ampullariella regularis*.[7,8] Neplanocin A, the most active antibiotic of this class, was a subject of several synthetic[9-12] and biochemical studies.[13-16] It was, therefore, of interest to prepare and examine open-chain analogues of neplanocin A. A recent preliminary announcement[17] of the synthesis and biological investigation of open-chain analogues of neplanocin A lacking the 2′-carbon unit (compounds **3a** and **3b**) has prompted us to report on our own studies of analogues **2a** and **2b**. Compound **2b** was also mentioned in two recent patent applications; however, no experimental data were provided.[18,19]

Results and Discussion

Synthesis. Alkylation of adenine (**5a**) or guanine precursor 2-amino-6-chloropurine (**5b**) with *trans*-1,4-dichloro-2-butene (**4**) was chosen as a simple and convenient approach to both open-chain analogues **2a** and **2b** (Scheme I). Alkylation at both ends of the halide **4** and lack of sufficient N^9/N^7 regioselectivity were considered at the outset as potential drawbacks to the method. However, these concerns proved groundless. Thus, reaction of adenine (**5a**) with halide **4** (4 equiv) in the presence of K_2CO_3 in Me_2SO[20] at room temperature gave a 60% yield of the desired chloro compound **6a** as a crystalline solid. Equally uncomplicated was alkylation effected by TBAF in THF,[21] which afforded intermediate **6a** in 70% yield. Reaction of 2-amino-6-chloropurine (**5b**) with halide **4** was also smooth, giving a 70–80% yield of alkylated product **6b** as a hygroscopic sirup by using either of the methods described above. The structures of intermediate **6a** and **6b** were confirmed by UV, mass, and ^1H NMR spectra. The latter showed that the regioselectivity of alkylation was 90–95%. This finding contrasts with some previous cases of alkylation of purine bases where the regioselectivity was 80% or lower.[22] Special precautions[22] (protecting groups and reagents) were reported to ensure regioselectivity of ca. 94%. It is also noteworthy that we have failed to detect any significant amount of α,ω-disubstitution product of the starting halide **4**. In the case of adenine (**5a**), no product of alkylation at N^3 was obtained, although similar compounds are readily formed in an uncatalyzed alkylation with reactive allyl halides, e.g., 1-bromo-3-methyl-2-butene.[23] In fact, yields of compounds **6a** and **6b** compare favorably with those obtained by alkylation of the sodium salt of adenine (**5a**) with a series of α-bromo-ω-chloroalkanes.[24] It should also be stressed that previous methods for synthesis of 9-(ω-hydroxyalkenyl)guanines are low yielding and require an unsymmetrically substituted alkylating agent.[19] Simple and rapid two-step syntheses of **2a** and **2b** described herein make use of an inexpensive α,ω-dichloroalkene as a starting material.

Structures of intermediates **6a** and **6b** were confirmed by UV, ^1H NMR, and mass spectra. The UV data, which are in agreement with those of appropriate model compounds,[25] are consistent with a predominant N^9-alkylation of both purines, **5a** and **5b**. ^1H NMR spectra (heterocyclic and CH_2 protons) of compounds **6a** and **6b** isolated by column chromatography indicated the presence of 5–10% of N^7-alkylated isomers. Although it was not possible to remove the latter contaminants by chromatography, crystallization of **6a** gave an isomerically pure compound. The absence of skeletal rearrangement or isomerization during alkylation was confirmed by ^1H NMR and mass spectra. The former indicated the presence of *trans*-CH=CH (J = 15 Hz) along with two CH_2 groups attached to a heteroatom (Cl or N) of δ 4.8–4.1. The mass spectra showed, in addition to molecular ions, the expected M – CH_2Cl fragments.

Intermediates **6a** and **6b** were transformed into analogues **2a** and **2b** by a simple hydrolysis. Thus, refluxing of crude **6a** in 0.1 M NaOH gave compound **2a** in 65% yield contaminated (^1H NMR) with ca. 10% of the 7-isomer, which was not possible to remove by column chromatography on silica gel. Nevertheless, pure analogue **2a**

(5) Suhadolnik, R. J. *Nucleoside Antibiotics*; Wiley-Interscience: New York, 1971; p 236.
(6) Larsson, A.; Alenius, S.; Johansson, N. G.; Öberg, B. *Antiviral Res.* **1983**, *3*, 77.
(7) Hayashi, M.; Yaginuma, S.; Muto, N.; Tsujino, M. *Nucleic Acids Res., Symp. Ser.* **1980**, *No. 8*, s 65.
(8) Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. *J. Antibiot.* **1981**, *34*, 675.
(9) Arita, M.; Adachi, K.; Ito, T.; Sawai, H.; Ohno, M. *J. Am. Chem. Soc.* **1983**, *105*, 4049.
(10) Jung, M.; Offenbächer, G.; Rêtey, *J. Helv. Chim. Acta* **1983**, *66*, 1915.
(11) Lim, M.-I.; Marquez, V. E. *Tetrahedron Lett.* **1983**, *24*, 5559.
(12) Tseng, C. K. H.; Marquez, V. E. *Tetrahedron Lett.* **1985**, *26*, 3669.
(13) Glazer, R. I.; Knode, M. C. *J. Biol. Chem.* **1984**, *259*, 12964.
(14) Keller, B. T.; Borchardt, R. T. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 131.
(15) Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. *J. Biol. Chem.* **1984**, *259*, 4353.
(16) Saunders, P. P.; Tan, M.-T.; Robins, R. K. *Biochem. Pharmacol.* **1985**, *34*, 2749.
(17) Haines, D. R.; Marquez, V. E.; Tseng, C. K. H.; Lee, W. J. 191st National Meeting of the American Chemical Society, New York, April 13–19, 1986; paper CARB 1.
(18) Hagberg, C.-E.; Johansson, K. N.-G.; Kovacs, Z. M. I.; Stening, G. B. European Patent 55 239; Bulletin 82/26, 1982.
(19) Johansson, K. N.-G.; Lindborg, B. G.; Noren, J.-O. European Patent 146 516; Bulletin 85/26, 1985.
(20) Montgomery, J. A.; Temple, C., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 630.
(21) Ogilvie, K. K.; Beaucage, S. L.; Gillen, M. F.; Entwistle, D. W. *Nucleic Acids Res.* **1979**, 6, 2261.
(22) Kjellberg, J.; Liljenberg, M.; Johansson, N. G. *Tetrahedron Lett.* **1986**, *27*, 877.
(23) Fujii, T.; Leonard, N. J. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, 1968; Vol. 1, p 13.
(24) Carraway, K. L.; Huang, P. C.; Scott, T. G. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Interscience: New York, 1968; Vol. 1, p 3.
(25) Žemlička, J. *Nucleosides Nucleotides* **1984**, *3*, 245.

was obtained by crystallization from acetone–methanol (7:3) in 46% overall yield. Similarly, refluxing of intermediate **2b** in 0.1 M HCl afforded crude compound **2b** in 70% yield. Again, pure analogue **2b** was obtained by crystallization from acetone–methanol–water (7:2:1) in 54% overall yield.

The isomeric purity and structural integrity of analogues **2a** and **2b** was verified by UV, ^{1}H NMR, and mass spectra. While it was not possible to ascertain the presence of CH_2OH in compound **2a** from the ^{1}H NMR spectrum because of overlapping OH and CH_2 signals, analogue **2b** exhibited OH as an expected triplet. In addition, mass spectra of both **2a** and **2b** showed prominent peaks of M – CH_2OH fragments.

Biological Activity. Compound **2a** is a substrate for adenosine deaminase. In that respect, it does resemble not only the "parent" neplanocin A[26] but also other open-chain analogues with adenine ring, such as **1b**[2] and (*S*)-9-(2,4-dihydroxybutyl)adenine.[25] The hydroxymethyl group of adenosine and related substrates is considered to be of utmost importance for a substrate binding to adenosine deaminase.[2,27] Apparently, the attachment of a similar function to an unsaturated center and the resultant restriction of rotational freedom does not prevent either neplanocin A[26] or analogue **2a** from acting as a substrate.

Compounds **2a** and **2b**, when tested as growth inhibitors of murine leukemia L 1210 cell culture, exhibited ID_{50} values of 1×10^{-3} M. No apparent inhibition of incorporation of labeled thymidine into DNA was detected. 1-β-D-Arabinofuranosylcytosine (*ara*-C), which was run for comparison, was active in 10^{-8} to 10^{-9} M range. Both analogues were inactive in antiviral assays against the following viruses: herpes simplex 1 (HSV 1), herpes simplex 2 (HSV 2), and vesicular stomatitis (VSV). No antiviral activity and cytotoxicity was observed up to 100–250 μg/mL. Further biological tests are in progress.

Experimental Section

General Procedures. All solvents and starting materials were of the highest available purity or they were purified as specified. Dimethyl sulfoxide (Me_2SO) was stored over Linde 3A molecular sieves. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ and it was stored over a sodium ribbon. Thin-layer chromatography was performed on 6 × 2 cm precoated aluminum sheets of silica gel 60 F_{254} (Merck) in the following solvents: (S_1) dichloromethane–ether (1:1), (S_2) dichloromethane–methanol (9:1), (S_3) dichloromethane–methanol (95:5), and (S_4) 2-propanol–NH_4OH–water (7:1:2). Kieselgel 60 (230–400 mesh ASTM, Merck) was used for column chromatography. Melting points were determined on a Thomas-Hoover apparatus and they are uncorrected. UV spectra were obtained with a Perkin-Elmer Lambda 5 spectrophotometer and IR spectra with a Perkin-Elmer 1330 infrared spectrophotometer. ^{1}H NMR spectra were determined with a General Electric QE-300 instrument. The spectra were run in $CDCl_3$ and dry CD_3SOCD_3 ("100 atom % D" in sealed ampules, Aldrich Chemical Co., Milwaukee, WI). Tetramethylsilane was used as an internal reference. The position of signals readily exchangeable with deuterium was verified by addition of D_2O to the sample in an appropriate solvent. Electron-impact mass spectra (MS) were measured with a Finnegan 4021 mass spectrometer.

9-(*trans*-4-Chloro-2-buten-1-yl)adenine (6a). A. With K_2CO_3 in Me_2SO. A mixture of adenine (**5a**; 0.54 g, 4 mmol), *trans*-1,4-dichloro-2-butene (**4**; 2 g, 16 mmol), and K_2CO_3 (freshly dried at 100 °C for 3 h, 1.11 g, 8 mmol) in Me_2SO (25 mL) was magnetically stirred for 5 h at room temperature. The progress of the reaction was followed by TLC in solvent S_2. The clear solution was evaporated in vacuo (oil pump), and the residue was dissolved in dichloromethane (100 mL). The insoluble portion was filtered off, it was washed with dichloromethane (15 mL), and the filtrate was evaporated. The residue was chromatographed on a silica gel column (60 g) in solvent S_1, which removed starting material **4**, followed by S_3, which eluted the product **6a** as the major component. Fractions containing **6a** were evaporated to give compound **6a**, 0.54 g (61%), homogeneous on TLC (S_2), mp 148 °C, after crystallization from ethyl acetate (148–150 °C): UV (ethanol) max 261 nm (ε 14300); ^{1}H NMR (CD_3SOCD_3) δ 8.13 and 8.10 (2 s, 2, adenine ring hydrogens), 7.24 (s, 2, NH_2), 6.05 and 5.65 (2 m, 2, *trans*-CH=CH, J = 15.2 Hz), 4.80 and 4.20 (2 d, 4, CH_2); MS, m/e (ion, relative abundance) 223 (M, 3.7), 188 (M – Cl, 36.3), 174 (M – CH_2Cl, 100.0), peaks of m/e 135, 108, 81, and 66 were also noted in the mass spectrum of adenine.[28] Anal. (C, H, Cl, N).

B. With Tetra-*n*-butylammonium Fluoride (TBAF) in THF. A mixture of adenine (**5a**; 0.54 g, 4 mmol), *trans*-1,4-dichloro-2-butene (**4**; 2 g, 16 mmol), and 1 M TBAF in THF (20 mL, 20 mmol) was magnetically stirred in THF (15 mL) for 16 h at room temperature. The clear solution was evaporated and the residue was chromatographed on a silica gel column (50 g) in solvent S_1 and then S_3 as eluents. The major UV-absorbing product which was homogeneous on TLC (S_2) was obtained by evaporation of appropriate fractions (0.64 g, 72%), mp 148 °C, identical with a sample of **6a** prepared by method A.

2-Amino-6-chloro-9-(*trans*-4-chloro-2-buten-1-yl)purine (6b). With K_2CO_3 in Me_2SO. The reaction was performed on the same scale as given for compound **6a** with 2-amino-6-chloropurine (**5b**). After 2 h at room temperature the mixture became clear and the reaction was complete. The solution was evaporated and the residue was washed with dichloromethane (3 × 40 mL). The solvent was evaporated and the crude product was chromatographed on a silica gel column (65 g) using solvent S_1. The latter removed excess of olefin **4** and elution with S_3 afforded TLC (S_2) homogeneous compound **6b** as a light yellow hygroscopic sirup (0.71 g, 69%): UV (ethanol) max 311 nm (ε 5000), 223 (ε 18000), 247 sh (ε 5000); ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1, purine ring H), 6.00 and 5.84 (2 m, 2, *trans*-CH=CH, J = 15 Hz), 5.35 (br s, 2, NH_2), 4.75 and 4.09 (2 d, 4, CH_2); MS, m/e (ion, relative abundance) 259, 258, 257, and 256 (M and M – 1, 3.3, 12.8, 3.7, and 19.3), 224 and 222 (M – Cl, 12.9 and 43.3), 210 and 208 (M – CH_2Cl, 24.4 and 72.5), 171 and 169 (purine base, 14.9 and 42.2), 134 (purine base – Cl, 51.5).

B. With TBAF in THF. The reaction with 2-amino-6-chloropurine (**5b**) was performed as described for preparation of adenine derivative **6a** (method B). Chromatography on silica gel afforded product **6b** (0.79 g, 79%), which was identical with the compound obtained by method A.

9-(*trans*-4-Hydroxy-2-buten-1-yl)adenine (2a). A solution of compound **6a** (1.12 g, 5 mmol) in 0.1 M NaOH (40 mL) was refluxed for 5 h. The mixture was evaporated, and the residue was coevaporated with methanol (20 mL), whereupon it was chromatographed on a silica gel column (60 g), using solvent S_2 as an eluent. Fractions containing **2a** were combined and evaporated to give a white solid, which was crystalized from an acetone–methanol (7:3) mixture (20 mL), giving 0.68 g (65%) of **2a**, mp 193–194 °C. Additional two recrystallizations afforded 0.47 g (46%): mp 200–201 °C; UV (0.01 M Na_2HPO_4, pH 7) max 261 nm (ε 14900); ^{1}H NMR (CD_3SOCD_3) δ 8.13 and 8.09 (2 s, 2, adenine ring hydrogens), 7.21 (s, 2, NH_2), 5.80 and 5.60 (2 m, 2, *trans*-CH=CH, J = 15.4 Hz), 4.75 and 3.90 (2 t, 5, OH and CH_2); MS, m/e (ion, relative abundance) 205 (M, 3.4), 188 (M – OH, 1.8), 174 (M – CH_2OH, 100.0), 148 (adenine CH_2, 4.7), peaks of m/e 135, 108, 81, 66, 54, and 28 were also found in the mass spectrum of adenine.[28] Anal. (C, H, N).

(26) Tsujino, M.; Yaginuma, S.; Fujii, T.; Hayano, K.; Matsuda, T.; Watanabe, T.; Abe, J. In *Current Chemotherapy and Infectious Disease*, Proceedings of the 11th International Congress of Chemotherapy; Nelson, J. D., Gassi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; Vol. 2, p 1559.

(27) Bloch, A.; Robins, M. J.; McCarthy, J. R., Jr. *J. Med. Chem.* **1967**, *10*, 908.

(28) Basile, B.; Scott, M. F.; Hsu, F.-F.; McCloskey, J. A. *Mass Spectra of Bases, Nucleosides, Nucleotides, and Their Derivatives*; University of Utah: Salt Lake City, UT, 1981; Vol. 1.

9-(*trans*-4-Hydroxy-2-buten-1-yl)guanine (2b). A solution of compound **6b** (1.29 g, 5 mmol) in 0.1 M HCl (30 mL) was refluxed for 5 h. The mixture was evaporated, and the residue was coevaporated with water (25 mL) and finally dissolved in the same solvent (30 mL). This solution was stirred with Dowex 1 (acetate, 10 g, wet weight) for 1 h at room temperature. The resin was filtered off, it was repeatedly washed with water (total of 1 L) and the filtrate was evaporated. The residue was chromatographed on a silica gel column (65 g) using solvent S_2 as an eluent. The appropriate UV-absorbing fractions were collected and evaporated to give a white solid, which was crystallized from an acetone–methanol–water (7:2:1) mixture (15 mL), giving 0.77 g (70%) of **2b**, mp 231–233 °C, homogeneous on TLC (S_4). Two more recrystallizations afforded 0.60 g (54%): mp 269 °C; UV (0.01 M Na_2HPO_4, pH 7) max 252 nm (ϵ 13 800), 272 sh (δ 10 200); NMR (CD_3SOCD_3) δ 10.65 (s, 1, purine ring NH), 7.63 (s, 1, purine ring H), 6.50 (s, 2, NH_2), 5.74 and 5.59 (2 td, 2, *trans*-CH=CH, J = 15 Hz), 4.75 (poorly resolved t, 1, OH), 4.54 and 3.89 (d and an apparent s, 4, CH_2); MS, m/e (ion, relative abundance) 221 (M, 1.6), 202 (1.4), 190 (M – CH_2OH, 4.8), peaks of m/e 151, 109, 69, 55, and 44 were found in the mass spectrum of guanine.[28] Anal. (C, H, N – hemihydrate).

Deamination of 9-(*trans*-4-Hydroxy-2-buten-1-yl)adenine (2a) with Adenosine Deaminase. Compound **2a** (0.5 mg, 2.4 μmol) and adenosine deaminase from calf intestine (type II, Sigma Chemical Co., St. Louis, MO, 0.4 units) were incubated in 0.05 M Na_2HPO_4 (pH 7.5, 0.4 mL) at room temperature with magnetic stirring. The final concentration of **2a** was 5.5×10^{-3} M. Aliquots were removed, and they were examined by TLC (S_2) and, after appropriate dilution, by UV spectroscopy. The reaction was complete after 19 h (UV max 250 nm). Adenine, which is a very weak substrate,[29] was completely resistant to deamination under the conditions of assay. By contrast, adenosine was quantitatively deaminated at 4.3×10^{-5} M with the same amount of enzyme in 4 min as shown by UV spectrophotometric assay.[30]

Acknowledgment. This investigation was supported by NIH Grant CA 32779 from the National Cancer Institute, Bethesda, MD, and by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. Thanks are due to the Central Instrumentation Facility, Department of Chemistry, Wayne State University, for measurements of ^1H NMR (Dr. O. Mols) and mass spectra (Dr. R. J. Hood). We also thank Dr. D. Kessel, Department of Pharmacology and Internal Medicine, Wayne State University School of Medicine, for assays in murine leukemia L 1210 cell culture and to Dr. H. E. Renis, Cancer Research, The Upjohn Company, Kalamazoo, MI, for antiviral screening. We also thank a referee for providing us with ref 18 and 19.

(29) Wolfenden, R.; Kaufman, J.; Macon, J. B. *Biochemistry* **1969**, *8*, 2412.
(30) Kaplan, N. O. *Methods Enzymol.* **1955**, *2*, 473.
(31) **Note added in proof**: Compounds **2a** and **2b** were inactive against vaccinia virus up to 320 μg/mL and HTLV-III (AIDS) virus (Canonico, P. G.; Broder, S., personal communications).

Synthesis and Antiviral Activity of Various 3′-Azido, 3′-Amino, 2′,3′-Unsaturated, and 2′,3′-Dideoxy Analogues of Pyrimidine Deoxyribonucleosides against Retroviruses[1]

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Various 3′-azido, 3′-amino, 2′,3′-unsaturated, 2′,3′-dideoxy, and 5-substituted analogues of pyrimidine deoxyribonucleosides have been prepared and tested against Moloney-murine leukemia virus (M-MULV), a mammalian T-lymphotropic retrovirus in vitro. Among these compounds, the 3′-azido analogues of thymidine, 2′-deoxy-5-bromouridine, and 2′-deoxy-5-iodouridine, the 2′,3′-unsaturated analogue of thymidine and 2′-deoxycytidine, and 2′,3′-dideoxycytidine were found to be most active, with ED_{50} values of 0.02, 1.5, 3.0, 2.5, 3.7, and 4.0 μM, respectively. These active compounds were nontoxic to the host SC-1 cells up to 100 μM concentration. The 3′-azido analogues of thymidine and 2′-deoxy-5-bromouridine were also tested in vitro against HTLV-III/LAV/AAV ("AIDS" virus) and found to be significantly active, with ED_{50} values of 0.23 and 2.3 μM, respectively. The structure–activity relationships are discussed.

There is a need for compounds that may be effective in the therapy of acquired immune deficiency syndrome (AIDS). It has been estimated that one million or more individuals in the United States have been exposed to the HTLV-III/LAV virus, the putative causative agent of AIDS, and as of May 1986 over 20 000 cases of AIDS have been reported. Of those diagnosed as having AIDS 3 or more years ago, 85% are now dead.

Some compounds have been identified as having an inhibitory effect against retroviruses, particularly the HTLV-III/LAV virus, and hence of potential use in the therapy of AIDS. These include HPA-23,[3,4] interferon,[5] ribavirin,[6] phosphonoformate,[7,8] ansamycin,[9] suramin,[10–12]

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(3) Dormont, D.; Spire, B.; Barre-Sinoussi, F.; Montagnier, L.; Chermann, J. C. *Ann. Inst. Pasteut/Virol.* **1985**, *136E*, 75.
(4) Rosenbaum, W.; Dormont, D.; Spire, B.; Vilmer, E.; Gentilini, M.; Griscelli, C.; Montagnier, L.; Barre-Sinoussi, F.; Chermann, J. C. *Lancet* **1985**, 450.
(5) Ho, D. D.; Hartshorn, K. L.; Rota, T. R.; Andrews, C. A.; Kaplan, J. C.; Schoolkey, R. T.; Hirsch, M. S. *Lancet* **1985**, 602.
(6) McCormick, J. B.; Getchell, J. P.; Mitchell, S. W.; Hicks, D. R. *Lancet* **1984**, 1367.
(7) Sandstrom, E. G.; Kaplan, J. C.; Byington, R. E.; Hirsch, M. S. *Lancet* **1985**, 1480.

0022-2623/87/1830-0440$01.50/0