# Carbocyclic Analogs of Guanosine and 8-Azaguanosine

**Y. FULMER SHEALY▲ and JOE D. CLAYTON**

**Abstract** □ (±)-9-[*trans*-2,*trans*-3-Dihydroxy-*cis*-4-(hydroxymethyl)-cyclopentyl]guanine, the carbocyclic analog of guanosine, was synthesized by a five-step route beginning with (±)-*trans*-3-amino-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol and 2-amino-4,6-dichloropyrimidine. Preparation and reduction of a 5-(*p*-chlorophenylazo)pyrimidine derivative introduced the prerequisite 5-amino substituent. Formation of the imidazole ring from this 5-amino derivative with triethyl orthoformate and acidic hydrolysis of the resulting mixture of 2-amino-6-chloropurine derivatives furnished the guanosine analog. The same 5-aminopyrimidine intermediate was diazotized to the 2-amino-6-chloro-8-azapurine, and the latter derivative was hydrolyzed to the carbocyclic analog of 8-azaguanosine. Neither of these analogs was cytotoxic to human epidermoid carcinoma cells *in vitro*. In tests using leukemia L-1210, the guanosine analog was quite toxic but was inactive at nontoxic doses; the 8-azaguanosine analog was not active in initial (single-dose) tests.

**Keyphrases** □ Guanosine, cyclopentyl analog—synthesis, cytotoxicity □ 8-Azaguanosine, cyclopentyl analog—synthesis, cytotoxicity □ Carbocyclic analogs of purine nucleosides—synthesis and cytotoxicity of cyclopentyl derivatives of guanine and 8-azaguanine □ Cyclopentyl derivatives of guanine and 8-azaguanine—synthesis, cytotoxicity □ Purine nucleosides, carbocyclic analogs—synthesis, cytotoxicity

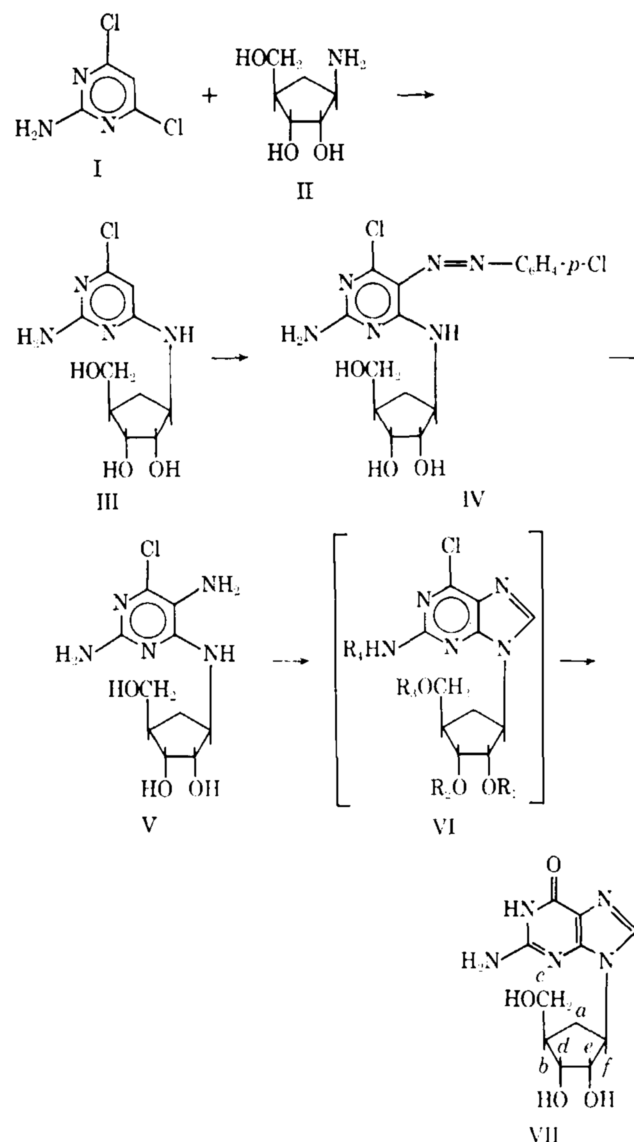
Syntheses of racemic, carbocyclic (cyclopentane) analogs of adenosine, 2′- and 3′-deoxyadenosine, adenylic acid, adenosine cyclic 3′,5′-monophosphate, and other 6-substituted purine nucleosides were described previously (1–4). Some of these nucleoside analogs, which have a stable carbon nitrogen bond at position 9 of the purine ring in place of the glycosidic bond, are cytotoxic to cells in culture (4–6), function as substrates for or inhibitors of purine-metabolizing enzymes (5–7), or display antimicrobial activity[1] (8). In the previously described analogs, the only substituent on the purine ring, other than the 9-cyclopentyl group, is at position 6. This report deals with the carbocyclic analogs of guanosine and 8-azaguanosine, which are 2-amino-6-substituted purines.

## DISCUSSION

The synthesis of the racemic guanosine analog is outlined in Scheme I. Prolonged treatment of 2-amino-4,6-dichloropyrimidine (I) with *trans*-3-amino-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (II) (2) gave the pyrimidinylaminocyclopentane derivative (III). Triethylamine was employed as a proton acceptor, rather than an excess of II, because of the difficulty in obtaining large amounts of II. Coupling of III with *p*-chlorobenzenediazonium chloride in aqueous acetic acid buffered with sodium acetate gave good yields of the 5-(*p*-chlorophenylazo)pyrimidine (IV). The required 5-amino group was introduced by reducing the *p*-chlorophenylazo group with zinc in acetic acid, a method previously found (9) to effect this type of reduction of simpler derivatives without significantly affecting the chloro substituent on the pyrimidine ring.

The purine ring was formed by an acid-catalyzed reaction of triethyl orthoformate with the tetrasubstituted pyrimidine (V). Reactions of the hydroxyl groups and the 2-amino group with the reagent should produce derivatives (formyl, orthoester, cyclic orthoester, or polymeric) of the expected 2-amino-6-chloropurine (VI, $R_1 = R_2 = R_3 = R_4 = H$). The isolation of pure derivatives (VI) was not attempted; instead, the material obtained from V and triethyl orthoformate was subjected to acidic hydrolysis to hydrolyze the 6-chloro substituent and to liberate the functional groups simultaneously. That purine ring formation had proceeded satisfactorily was shown by the 78% overall yield of the guanosine analog (VII) from V. The proton magnetic resonance (PMR) and the UV absorption spectra were in accord with Structure VII.

Treatment of pyrimidine V (Scheme II) with sodium nitrite and aqueous acetic acid produced a high yield of the 5-amino-7-chloro-3*H*-*v*-triazolo[4,5-*d*]pyrimidine (VIII). Either acidic or basic hydrolysis of VIII gave the 8-azaguanosine analog (IX).

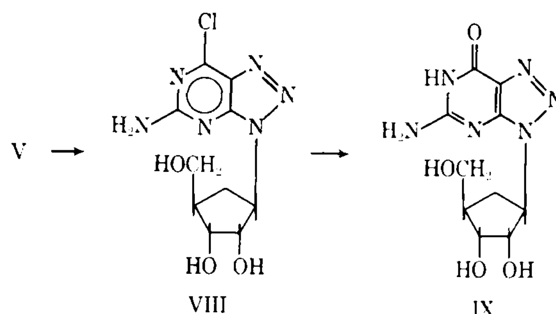



*Scheme I—Letters* a–f *identify protons with proton magnetic resonance chemical shifts* (*see* Experimental)

[1] R. F. Pittillo, Southern Research Institute, Birmingham, Ala., personal communication.

The guanosine analog (VII) was tested against leukemia L-1210 in mice on three treatment schedules[2]. When administered on Day 1 at doses of 400, 300, 150, and 75 mg./kg., VII displayed acute toxicity at the two higher doses. The difference ($\Delta W$) between the average weight changes of the treated (*T*) and the untreated (*C*) mice indicated that the two lower doses were also toxic ($\Delta W$ = −5.6 and −3.9 g., respectively). Administered on Days 1, 5, and 9, doses of 38 and 19 mg./kg. were nontoxic and inactive; a shortened survival time ($T/C$ = 70%) of treated animals indicated that a dose of 75 mg./kg. on this schedule is toxic. Doses of 38 and 19 mg./kg./day given *q.d.* 1–9 were toxic, as evidenced by shortened survival times ($T/C$ = 71 and 83%, respectively) relative to untreated animals and by weight differences ($\Delta W$ = −5.1 and −2.3 g., respectively), whereas doses of 9.5 and 5 mg./kg./day were nontoxic and inactive. These data, especially the results of daily treatment, indicate that the toxicity of VII *in vivo* is approximately the same as that of the adenosine analog (4). In contrast to the similarity of the toxicity of the two analogs *in vivo*, the guanosine analog (VII) is not cytotoxic ($ED_{50}$ >100 mcg./ml.) to human epidermoid carcinoma cells (H.Ep.-2) in culture, whereas the adenosine analog is highly cytotoxic[3] ($ED_{50}$ = 0.7 mcg./ml.) (4).

The 8-azaguanosine analog (IX) was not cytotoxic ($ED_{50}$ >100 mcg./ml.) to H.Ep.-2 cells in culture and was not active against L-1210 leukemia at doses of 100, 200, or 400 mg./kg. administered on the Day 1 schedule. The observed $\Delta W$ (−2.3 g.) at the highest dose was indicative of chronic toxicity.




*Scheme II*

## EXPERIMENTAL[4]

**(±)-*trans*-3-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (III)**—A mixture of 3.68 g. (22.4 mmoles) of 2-amino-4,6-dichloropyrimidine (I), 3.3 g. (22.4 mmoles) of *trans*-3-amino-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (II) (2), 2.26 g. (22.4 mmoles) of triethylamine, and 200 ml. of absolute ethanol was heated under reflux for 140 hr. The white, crystalline product was separated by filtration from the cooled reaction mixture, yielding 3.9 g. (63%), m.p. 234–238° dec. A specimen was recrystallized from water, m.p. 236–239° dec.; UV: $\lambda_{max}$ 302 (sh), 287 (infl), 274 ($\epsilon$ 9600), 240 ($\epsilon$ 12,600), and 214 ($\epsilon$ 19,800) at pH 1; 286 ($\epsilon$ 10,200) and 237 ($\epsilon$ 11,400) at pH 7; and 287 ($\epsilon$ 10,200) and 238 ($\epsilon$ 11,200) at pH 13.

*Anal.*—Calc. for $C_{10}H_{15}ClN_4O_3$: C, 43.72; H, 5.51; N, 20.40; Cl, 12.90. Found: C, 43.53; H, 5.49; N, 20.20; Cl, 12.9.

**(±)-*trans*-3-{[2-Amino-6-chloro-5-[(*p*-chlorophenyl)azo]-4-pyrimidinyl]amino}-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (IV)**—A cold (0–5°) solution of *p*-chlorobenzenediazonium chloride, prepared from 5.90 g. (46.3 mmoles) of *p*-chloroaniline, 12.8 g. of 12 *N* HCl, 52 ml. of water, and 3.5 g. (50.5 mmoles) of sodium nitrite in 42 ml. of water, was added dropwise during 1 hr. to a well-stirred mixture of 11.53 g. (42 mmoles) of III, 84 g. of sodium acetate trihydrate, 200 ml. of acetic acid, and 200 ml. of water. The diazonium salt solution was maintained at 0–5° by adding it from a jacketed addition funnel kept cold with circulating ice water; the pyrimidine (III) solution was at room temperature. The mixture was stirred for 18 hr. at room temperature, cooled in an ice bath, and filtered to remove the yellow precipitate, which was washed with cold water and dried *in vacuo* over phosphorus pentoxide, yielding 14.1 g. (81%), m.p. 249–250° dec. (inserted at 230°); homogeneous by TLC [10 mcg., chloroform–methanol (5:1)]. After several days at room temperature, the filtrate deposited a second crop, weighing 1.25 g., m.p. 245–246° dec. A specimen of IV for analysis was recrystallized from dimethylformamide–water, m.p. 258–259° dec. (inserted at 240°, 2°/min.) and m.p. 262–263° dec. (inserted at 250°, 2°/min.); UV: $\lambda_{max}$ 372 ($\epsilon$ 26,600), 280 ($\epsilon$ 8800), and 240 ($\epsilon$ 18,300) at pH 1; and 385 ($\epsilon$ 28,600), 280 ($\epsilon$ 10,700), and 227 ($\epsilon$ 16,300) at pH 7 and 13.

*Anal.*—Calc. for $C_{16}H_{18}Cl_2N_6O_3$: C, 46.50; H, 4.39; Cl, 17.16; N, 20.34. Found: C, 46.33; H, 4.29; Cl, 17.3; N, 20.32.

**(±)-*trans*-3-[(6-Chloro-2,5-diamino-4-pyrimidinyl)amino]-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (V)**—A mixture of 7.2 g. of the *p*-chlorophenylazopyrimidine (IV), 140 ml. of ethanol, 172 ml. of water, and 14 ml. of acetic acid was heated to 70° with vigorous mechanical stirring under a nitrogen atmosphere. Zinc dust (11.2 g.) was added in small portions during 45 min., and the mixture was heated at 70° with vigorous stirring for an additional 1.25 hr. Excess zinc was filtered from the hot mixture and washed with three portions of ethanol; the filtrate, combined with the ethanol washings, was concentrated *in vacuo* to about 50 ml.; and the resulting mixture was diluted with ethanol (50 ml.) and stored at 5° overnight. The tan crystalline precipitate was separated by filtration, washed with ethanol–water (3:1), and dried *in vacuo* at 78°, yielding 2.46 g. (49%), m.p. 245–247° dec. (inserted at 230°); UV: $\lambda_{max}$ 298 ($\epsilon$ 8000), 237 ($\epsilon$ 15,500), and 210 ($\epsilon$ 16,200) at pH 1; 303 ($\epsilon$ 9000), 240 (sh), 225 (sh), and 204 ($\epsilon$ 19,200) at pH 7; and 303 ($\epsilon$ 9000), 240 (sh), and 225 (sh) at pH 13.

*Anal.*—Calc. for $C_{10}H_{16}ClN_5O_3$: C, 41.45; H, 5.57; Cl, 12.24; N, 24.18. Found: C, 41.22; H, 5.70; Cl, 12.4; N, 24.00.

The filtrate was concentrated *in vacuo*. A water (100 ml.) solution of the residual oil was washed with ether (3 × 75 ml.), concentrated *in vacuo* to about 30 ml., and kept at 5° overnight. Recrystallization of the brown crystalline precipitate from water gave 320 mg. of V (total yield 55%), m.p. 235–243° dec.

**(±)-9-[*trans*-2,*trans*-3-Dihydroxy-*cis*-4-(hydroxymethyl)cyclopentyl]guanine (VII)**—A solution consisting of 1.45 g. of V, 20 ml. of dimethylformamide, 40 ml. of triethyl orthoformate, and 1 ml. of 12 *N* HCl was kept at room temperature for 1.5 hr., at 5° for 16 hr., and at room temperature for 8 hr. The reaction solution was concentrated *in vacuo* at 30°. A mixture of the residual syrup and 200 ml. of 2 *N* HCl was heated under reflux for 5 hr. and then concentrated *in vacuo* at 50°. Several portions of water were evaporated *in vacuo* from the residue before it was dissolved in water (10 ml.). The pH of the aqueous solution was adjusted to 5 with 6 *N* NaOH, the mixture was chilled (5°) for 2 hr., and the solid precipitate (1.285 g.) was separated by filtration and washed with water. The precipitate was recrystallized from water (15 ml.) and dried *in vacuo* over phosphorus pentoxide at 78° for 18 hr., yielding 1.154 g. (78% as a 0.75 hydrate), m.p. 279–282° dec. (softened and resolidified in 250–260° range); purity 96–97% by UV (calculated as a 0.75 hydrate). A sample for analysis [white needles (150 mg.) obtained by recrystallizing 225 mg. of crude VII from 10 ml. of water] was dried at 100° over phosphorus pentoxide for 20 hr., m.p. 278–284° dec. (inserted at 230°, softened and darkened at 250–255°, resolidified);

[2] In the standard L-1210 leukemia test, mice are innoculated intraperitoneally on Day 0 with $10^5$ L-1210 cells. Administration of a compound is also by intraperitoneal injection. Weight changes are determined 4 days after the first (or only) injection. Weight differences in excess of 4 g. or values of $T/C \lesssim 85\%$ are accepted criteria of chronic toxicity. Since the carbocyclic analogs have generally shown a good correlation between dose and weight difference ($\Delta W$), a weight difference of 2–3 g. also appears to indicate chronic toxicity.

[3] $ED_{50}$ = concentration of a compound that inhibits growth, measured by protein determinations, to 50% of the growth of untreated cells.

[4] Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus and are not corrected. UV spectra were recorded with a Cary model 17 or 14 spectrophotometer. UV maxima are in nanometers; sh = shoulder, and infl = inflection. Solutions for UV determinations were prepared by diluting a 5-ml. aliquot of a water or ethanol solution (0.1 *N* HCl for III) to 50 ml. with 0.1 *N* HCl, phosphate buffer (pH 7), or 0.1 *N* NaOH; absorption maxima are reported at pH 1, 7, or 13. IR spectra were recorded with a Perkin-Elmer model 621 or 521 spectrometer from samples in KBr disks. PMR spectra were determined with a Varian model XL-100-15 spectrometer for observing proton resonance at 100 MHz. Chemical shift data ($\delta$) are in parts per million downfield from tetramethylsilane, the internal reference; s = singlet, d = doublet, and m = multiplet. Unless otherwise indicated, TLC was performed on plates of silica gel, and spots were detected by: (*a*) UV light (254 nm.) after spraying the chromatogram with an optical whitening agent (Ultraphor WT, BASF Colors and Chemicals, Inc., Charlotte, N. C.) and (*b*) spraying with a basic solution of potassium permanganate. The quantity applied and the developing solvent are shown parenthetically at the appropriate places in the procedures.

IR: 1725, 1675, 1625, 1605 (sh), 1565, 1545, 1480, 1170, 770 (sharp), and 680 cm.$^{-1}$ and other bands (broad or weak); PMR[5] (dimethyl sulfoxide-$d_6$): $\delta$ about 1.3–1.7 (m, H*a*), about 1.8–2.4 (m, H*a*H*b*), 3.28–3.6 (m, H*c*H*c*), 3.37 (s, water), 3.72–3.9 (m, H*d*), 4.08–4.34 (m, H*e*), 4.36–4.78 (m, H*f* + 2OH), 4.82–4.98 (d, OH), 6.39 (s, $NH_2$), 7.79 (s, $H_8$ of purine ring), and 10.58 (s, NH of purine ring); UV: $\lambda_{max}$ ($\epsilon$ calculated for 0.75 hydrate) at 278 ($\epsilon$ 8000) and 255 ($\epsilon$ 11,900) at pH 1; 268 (sh) and 253 ($\epsilon$ 13,000) at pH 7; and 268 ($\epsilon$ 11,100) and 257 (sh) ($\epsilon$ 10,500) at pH 13.

*Anal.*—Calc. for $C_{11}H_{15}N_5O_4 \cdot {}^3/_4H_2O$: C, 44.81; H, 5.64; N, 23.76. Found: C, 44.75; H, 5.87; N, 23.44.

Specimens of VII dried at 100° for about 20 hr. apparently rehydrated readily since they were observed to increase in weight during weighing for analysis. The melting point was evidently influenced by the degree of hydration as well as the heating rate. A specimen recrystallized from dimethylformamide ethanol melted at 298–300° dec. without prior softening (inserted at 100°, 2°/min. at 260°). Recrystallization of this specimen from water (including treatment with activated carbon) changed the melting point to 284–286° dec. (softened and darkened at 260°, resolidified; inserted at 230°, 2°/min. at 260°); homogeneous by TLC [silica gel or cellulose, 10 mcg., propanol water (3:1); detection by UV, UV and spraying[6], and potassium permanganate spray]. The exact values of $\epsilon$ are also determined by the degree of hydration at the time the sample is weighed for UV determination.

**(±)-*trans*-3-(5-Amino-7-chloro-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (VIII)** A solution of 240 mg. (3.48 mmoles) of sodium nitrite in 6 ml. of water was added dropwise to a cold (0°) mixture of 870 mg. (3.0 mmoles) of V, 9 ml. of water, and 3 ml. of acetic acid. The mixture was stirred at 0° for 1 hr., and the product was separated by filtration and washed with 5 ml. of cold water, yielding 828 mg. Recrystallization of the crude VIII from ethanol hexane gave a white solid, which was dried *in vacuo* over phosphorus pentoxide at room temperature for 18 hr. and then at 78° for 0.5 hr., yielding 766 mg. (85%), m.p. 172–174° dec.; TLC: 1 spot [20 mcg., chloroform–methanol (4:1)]; UV: $\lambda_{max}$ 226 ($\epsilon$ 21,600), 245–260 (infl), and 316 ($\epsilon$ 7500) at pH 1; 226 ($\epsilon$ 21,900), 245–260 (infl), and 316 ($\epsilon$ 7500) at pH 7; and 246 ($\epsilon$ 4700) and 286 ($\epsilon$ 9900) at pH 13.

*Anal.*—Calc. for $C_{10}H_{13}ClN_6O_3$: C, 39.94; H, 4.36; N, 27.95. Found: C, 39.75; H, 4.55; N, 27.71.

**(±)-5-Amino-3,6-dihydro-3-[*trans*-2,*trans*-3-dihydroxy-*cis*-4-(hydroxymethyl)cyclopentyl]-7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one (IX)**— A mixture of 250 mg. of VIII and 20 ml. of 1 *N* HCl was heated under reflux for 4 hr. and concentrated *in vacuo* at 40° to a syrup. Water (2 × 20 ml.) was added to the residue and evaporated *in vacuo*, and ethanol (10 ml.) was then added. A gelatinous solid was separated by filtration, washed with acetone, and dried *in vacuo* over phosphorus pentoxide at 78°, yielding 75 mg. The filtrate and the acetone washings were combined and evaporated *in vacuo*, the residue was triturated with ethanol–hexane (1:1, 10 ml.), and a gelatinous solid was filtered away and dried *in vacuo* over phosphorus pentoxide at 78°, yielding 99 mg. (total yield 74%). Both portions were homogeneous according to TLC [20 mcg., ethanol–15 *N* aqueous ammonia (4:1)]. A 6 *N* HCl solution of the two portions was diluted with ethanol, and the gelatinous precipitate was dried as before, yielding 88 mg. (38%), m.p. 252–254° dec. (inserted at 240°, 2°/min.); IR: 1700, 1635, 1595 (sh), 1580, 1525, 780, and 680 cm.$^{-1}$; UV: $\lambda_{max}$ 253 ($\epsilon$ 11,800) and 270 (sh) at pH 1, 253 ($\epsilon$ 11,700) and 270 ($\epsilon$ 8600) at pH 7, and 279 ($\epsilon$ 11,000) and 260 (sh) at pH 13.

*Anal.*—Calc. for $C_{10}H_{14}N_6O_4 \cdot {}^2/_3H_2O$: C, 40.81; H, 5.25; N, 28.56. Found: C, 40.99; H, 5.22; N, 28.60.

Hydrolysis of VIII with refluxing 1 *N* NaOH also yields IX.

[5] The positions of protons *a–f* are shown in Structure VII.
[6] Ultraphor WT.

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▲ To whom inquiries should be directed.