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# Synthesis of Cyclohexyl Carbocyclic Puromycin and Its Inhibition of Protein Synthesis

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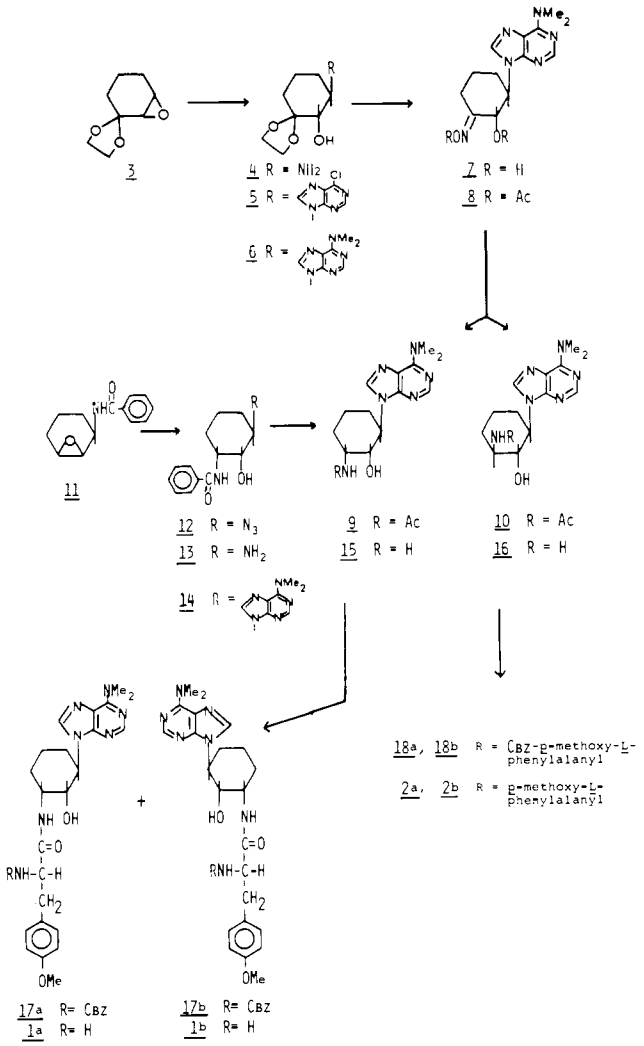
In a continuation of our studies with puromycin analogues, four cyclohexyl carbocyclic puromycins, 6-dimethylamino-9-[(*R*)-[2(*R*)-hydroxy-3(*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (**1a**), 6-dimethylamino-9-[(*S*)-[2(*S*)-hydroxy-3(*S*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (1b), 6-dimethylamino-9-[(*R*)-[2-(*R*)-hydroxy-3(*S*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (**2a**), and 6-dimethylamino-9-[(*S*)-[2(*S*)-hydroxy-3(*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (**2b**), have been synthesized. *trans*-3-Amino-2-hydroxycyclohexanone ethylene ketal (**4**) was easily obtained by opening of epoxide **3** with liquid ammonia. Condensation of **4** with 5-amino-4,6-dichloropyrimidine and subsequent ring closure gave the 9-substituted 6-chloropurine **5** which was converted to the *O*-acetyloxime **8**. Reduction of **8** with diborane gave a mixture of *cis*- and *trans*-amino alcohols separated as their acetamides, **9** (7%) and **10** (33%), respectively. The amino alcohols were converted to **1a**,b and **2a**,b by a general method previously reported from our laboratory. In addition, the *trans*-cyclopentyl carbocyclic puromycins, 6-dimethylamino-9-[(*R*)-[2(*R*)-hydroxy-3(*S*)-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl]purine and 6-dimethylamino-9-[(*S*)-[2(*S*)-hydroxy-3(*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl]purine (**19a** and **19b**), were prepared from the previously described 2$\alpha$-acetamido-5$\alpha$-(6-dimethylamino-9-purinyl)cyclopentan-1$\beta$-ol. The puromycin analogues were evaluated as inhibitors of protein synthesis by their ability to inhibit the rate of poly(U,C)-directed L-[$^{14}$C]poly(phenylalanine) formation in an *Escherichia coli* cell-free ribosome system. The results of this study, in conjunction with our previous reports, suggest that maximum activity of puromycin analogues is obtained when the purine moiety, the amino acid, and the hydroxyl group are oriented about a five-membered ring. In addition, the amino acid and hydroxyl group must be in a cis orientation, and the absolute stereochemistry of the parent antibiotic must be conserved.

Previous reports relating to our studies on puromycin analogues have described the antimicrobial[1] and antitumor activity[2] of a carbocyclic puromycin analogue (**20a**) in which the furanosyl ring was replaced with a cyclopentyl moiety. In vitro testing demonstrated that **20a** inhibits the formation of poly(phenylalanine) in the *Escherichia coli* cell-free system[3] and that its mechanism of action involves the termination of protein synthesis by accepting the growing peptide chain from peptidyl-tRNA.[4] The inhibition is stereospecific with the diastereomer **20b** being only slightly active.

The relative ease of preparing the corresponding cyclohexyl series of intermediates has prompted the synthesis of cyclohexyl carbocyclic puromycins **1a**,**b** and **2a**,**b**. The synthesis followed a route similar to that previously described for the synthesis of the cyclopentyl analogues of puromycin[1] (Scheme I). The amino alcohol **4** was easily obtained by opening of the epoxide **3** with liquid ammonia. Condensation of **4** with 5-amino-4,6-dichloropyrimidine and subsequent ring closure with triethyl orthoformate gave the 9-substituted 6-chloropurine **5**, which was converted to the corresponding 6-dimethylaminopurine **6**. The ketal of **6** was hydrolyzed in the presence of hydroxylamine to give oxime **7**. The *O*-acetyloxime **8** was reduced with diborane to a mixture of amino alcohols and separated chromatographically as their acetamides, **9** (7%) and **10** (33%). The ratio of **9** to **10** is in contrast to that previously reported for the diborane reduction of the analogous cyclopentyl compound, where the *cis*-acetamido alcohol was the major product (45%) and the *trans*-acetamido alcohol was the minor product (4%).[1] It has been noted in the reduction of acetylated 2-oximino-$\alpha$-D-hexopyranosides that there is an axially directed nucleophilic attack by the borane complex at C-2.[5] It is not surprising that the reduction of **8** gives the equatorial amine (*trans*-amino alcohol) as the major product. Possibly, in the case of the cyclopentyl analogue,[1] a borane complex at N-3 of the purine ring effects the reduction of the *O*-acetyloxime resulting in hydride attack selectively from the purine side of the ring. This situation would not be sterically feasible in the cyclohexyl analogue where the N-3 of the pseudoequatorial purine is quite far from C-3. It is also possible that hydride attack in the cyclopentyl case is directed primarily to the purine side of the ring by the 2-acetoxy group for steric reasons.

The structural assignments of **9** and **10** were confirmed by an alternate synthesis of **9**. Epoxide **11**[6] was opened with sodium azide and the azido alcohol **12** was obtained (87%). Catalytic reduction of **12** gave amine **13** (91%). The purine moiety was formed in the usual way by condensation of **13** with 5-amino-4,6-dichloropyrimidine, followed by ring closure with triethyl orthoformate to the 9-substituted 6-chloropurine. Aqueous dimethylamine was used to convert the chloropurine to the 6-dimethyl-

Scheme I



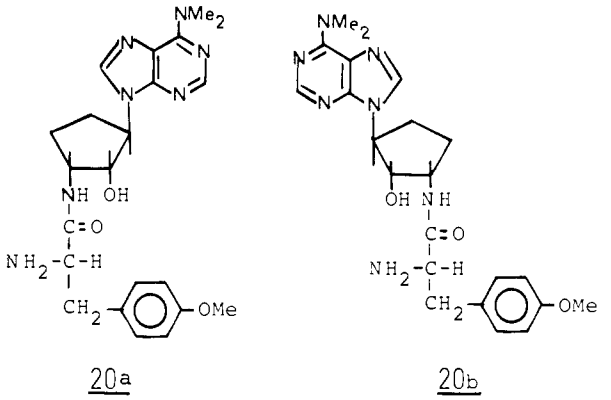

aminopurine 14 (60% from 13). The benzamide group of 14 was hydrolyzed in refluxing barium hydroxide, giving amine 15, which was unstable in air. A sample of 15 was acetylated to 9, thus confirming the structural assignment of the isomers isolated from the diborane reduction of 8.

The amine 15 was coupled with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamine by the *N*-hydroxysuccinimide–dicyclohexylcarbodiimide method[1] (Scheme I). A 1:1 diastereomeric mixture of **17a** and **17b** was obtained. Following hydrogenolysis of the *N*-benzyloxycarbonyl group of **17a** and **17b**, the diastereomers were separated chromatographically, giving pure **1a** and **1b**. Structure **1a** is assigned to the diastereomer having $[\alpha]^{25}_D$ of −99.7° and structure **1b** to the diastereomer having $[\alpha]^{25}_D$ of +21.1°).

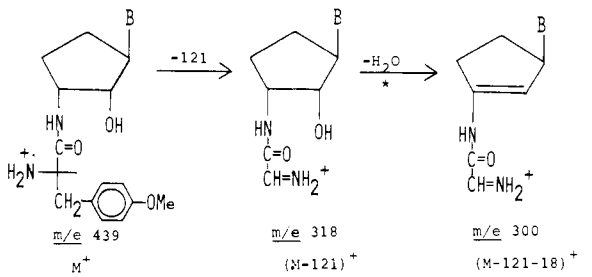
Acetamide **10** was hydrolyzed to amine **16**, a stable crystalline compound (Scheme I). Coupling of **16** with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine gave a mixture of **18a** and **18b**. Hydrogenolysis of this mixture gave **2a** and **2b**, separated chromatographically. Because of the relative inactivity of these isomers in a biological system, no attempt was made to determine absolute stereochemistry.

The *trans*-cyclopentyl compounds **19a** and **19b** were prepared from 2α-acetamido-5α-(6-dimethylamino-9-purinyl)cyclopentan-1β-ol[1] as described for the cyclohexyl compounds.

An interesting major difference between the mass spectra of the cis isomers **1a,b** and **20a,b** and the trans isomers **2a,b** and **19a,b** was observed. The mass spectra of all the compounds are dominated by fragmentation of the aminoacyl moiety. As reported earlier,[1] cleavage of the benzylic bond accounts for a prominent peak at *m/e* 318 (M − 121) in the spectra of the *cis*-cyclopentyl compounds **20a,b**. A large metastable peak indicated that loss of $H_2O$




from the (M − 121) ion resulted in the (M − 121 −18) ion, which is the base peak. Both the (M − 121) and (M − 121 − 18) ions are also very prominent in the spectra of the *cis*-cyclohexyl compounds. In contrast, all of the trans compounds show a strong (M − 121) peak but an extremely low intensity (M − 121 − 18) peak. It would appear that loss of $H_2O$ from the (M − 121) ion occurs by trans elimination and thus is favorable only for the cis isomers. This difference in the ease of loss of $H_2O$ is also noted for the $M^+ \rightarrow (M - 18)^+$ transition in the spectra of the acetamides **9** and **10** and the corresponding cyclopentyl




acetamides previously reported. Under the same conditions (probe temperature 125 °C, 20 eV), **9** showed an (M − 18) peak of relative intensity 6.6%, while the (M − 18) peak of **10** was 0.5%.

**Biological Activity.** The puromycin analogues were evaluated as inhibitors of protein synthesis by their ability to inhibit the rate of poly(U,C)-directed L-[$^{14}$C]poly(phenylalanine) formation in an *E. coli* cell-free ribosome system. The preference for the use of poly(U,C) instead of poly(U) as the mRNA for this assay has been described by us in a previous report.[9a] Also, it has very recently been reported that synthesis directed by poly(U,C) is more sensitive to inhibition by puromycin than poly(U)-directed synthesis and frequently shows a sensitivity to puromycin similar to that directed by endogenous mRNA.[9b] As expected, a cis orientation of the aminoacyl moiety and the hydroxyl group on the carbocyclic ring was required for activity. The trans isomers **2a,b** and **19a,b** were inactive at a concentration of $10^{-3}$ M. The inhibition of protein synthesis by the cyclohexyl carbocyclic puromycin analogue **1a** is presented in Figure 1. The activity is **1a** is compared with the activity profiles of the cyclopentyl analogue **20a** and puromycin. Examination of the 50% inhibitory concentrations from Figure 1 reveals that a 45-fold reduction in activity results from the substitution of a cyclopentyl ring with a cyclohexyl ring. Previous

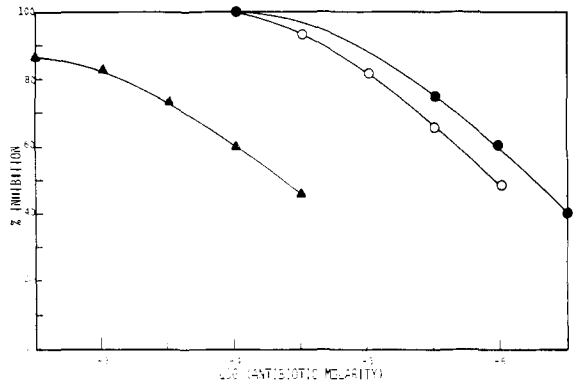


**Figure 1.** Inhibition of poly(U,C)-directed L-[$^{14}$C]poly(phenylalanine) formation in an *E. coli* cell-free ribosome system by puromycin (●), **20a** (○), and **1a** (▲). Assay conditions were as described in the Experimental Section.

studies with freely rotating acyclic puromycin analogues have demonstrated the requirement for the more conformationally rigid system provided by a ring system.[7] The decreased activity of the cyclohexyl analogues further establishes the rigid conformational requirements provided by the five-membered ring.

The diastereoisomer **1b** was inactive at $10^{-3}$ M. This observation is consistent with the previous observation that diastereoisomer **20b** is relatively inactive.[1] Stereochemistry of the active isomer was tentatively assigned that of structure **20a** on the basis of its biological activity and in accordance with the stereochemistry of puromycin.[1] This assignment has since been confirmed by x-ray analysis[8] of **20b**. The assignment of structure **1a** to the active isomer in the cyclohexyl series is consistent with our previous assignment of **20a**. The results of this study, in conjunction with our previous reports,[1,7] suggest that maximum activity of puromycin analogues is obtained when the purine moiety, the amino acid, and the hydroxyl group are orientated about a five-membered ring. In addition, the amino acid and hydroxyl group must be in a cis orientation, and the absolute stereochemistry of the parent antibiotic must be conserved.

## Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected; IR, in KBR disks, with a Perkin-Elmer 237B spectrophotometer; $^1$H NMR, with a Varian A-60D spectrometer using $Me_4Si$ as an internal standard. Analytical TLC was run on silica gel (Eastman chromagram sheets with fluorescent indicator); preparative TLC was done on 20 × 20 cm glass plates coated with 2 mm of silica gel $F_{254}$ (E. Merck, Darmstadt). Evaporations were carried out in vacuo with a bath temperature of less than 50 °C. Samples were dried in vacuo (<1 mm) at 56 °C before analysis. Mass spectra were run on an AEI MS-30, double beam, double focusing mass spectrometer with an AEI DS-30 data system. The polynucleotides were purchased from Miles Laboratories and ATP, GTP, phosphoenol pyruvate, and pyruvate kinase were purchased from Sigma. Puromycin dihydrochloride was obtained from Nutritional Biochemicals, [$^{14}$C]-L-phenylalanine was purchased from New England Nuclear, and tRNA was purchased from Calbiochem. *E. coli* ribosomes and S-100 fraction were prepared as previously described.[9]

**Inhibition of [$^{14}$C]-L-Poly(phenylalanine) Formation.** Reactions were performed in a final volume of 50 μL and contained 0.1 M Tris–Cl (pH 8), 0.05 M KCl, 0.01 M β-mercaptoethanol, 0.005 M phosphoenol pyruvate, 0.001 M ATP, 0.05 mM GTP, 30 μg/mL of pyruvate kinase, 4 μL (40 μg of protein) of S-100 fraction, 0.5 μCi/mL of [$^{14}$C]-L-phenylalanine (no other amino acids present), 15 mM $Mg(OAc)_2$, 1.2 mg/mL of ribosomes, and 2.0 μm of P/mL of poly(U,C) (1:1). In a typical experiment a solution was made which contained all of the above components, except poly(U,C) and the desired concentration of inhibitor. The reaction was initiated by the addition of poly(U,C). Incubations were for 15 min at 37 °C.

The reaction is terminated by the addition of 0.2 mL of 0.5 N NaOH and the mixture is incubated for 15 min at 37 °C. Each tube is neutralized with 0.2 mL of 0.5 N HCl and then placed in an ice bath. To each tube is added 2 mL of 10% cold trichloroacetic acid (TCA). After 5 min the solutions were filtered through type HA, 0.45-μ pore size Millipore filters with 2 × 3 mL washes with cold 5% TCA. The filters were dried under heat lamps and placed in counting vials with a toluene–Permablend liquid scintillator and counted in a Beckman LS-150. Counting efficiency was 85–90%. All counts were corrected by blanks in which poly(U,C) was absent. All values represent an average of triplicate determinations. The standard deviation of such replicates averaged <±6%.

**7-Oxabicyclo[4.1.0]heptan-2-one Ethylene Ketal (3).** Cyclohex-2-enone ethylene ketal[10] was allowed to react with NBS in $Et_2O$–$H_2O$ buffered by $NaHCO_3$ as described previously,[1] giving 2-bromo-3-hydroxycyclohexanone ethylene ketal as white granules from EtOAc–hexanes (76%): mp 97–98 °C; IR and $^1$H NMR as expected. Anal. ($C_8H_{13}BrO_3$) C, H. The crude bromohydrin was best dehydrohalogenated immediately, without purification, as it darkened rapidly. Crude 2-bromo-3-hydroxycyclohexanone ethylene ketal (15.3 g, 64.4 mmol), NaOH (powdered, 5.2 g, 0.13 mol), and benzene (125 mL) were refluxed for 1.0 h. The mixture was cooled, filtered, and concentrated to a pale yellow liquid which was distilled to give **3** as a colorless liquid (93%): bp 80–81 °C (2 mm); $n^{20}_D$ 1.4790; IR (neat) 1200–1040 (ketal), 1250, 865, 770 cm$^{-1}$ (epoxide); $^1$H NMR ($CCl_4$) δ 3.98 (m, $OCH_2CH_2O$), 3.20 (m, H-6), 2.78 (half of AB q, $J$ = 4.0 Hz, H-1), 1.9–1.2 (m, $3CH_2$). Anal. ($C_8H_{12}O_3$) C, H.

***trans*-3-Amino-2-hydroxycyclohexanone Ethylene Ketal (4).** Epoxide **3** (10.0 g, 64.0 mmol) in liquid $NH_3$ (40 mL) was maintained at 60–70 °C for 48 h in a steel bomb. The $NH_3$ was allowed to evaporate, and the white solid residue crystallized from benzene, giving **4** as white needles (7.89 g, 71%): mp 119.5–121 °C; IR (Nujol) 3345, 3265, 1575 ($NH_2$), 3200 (OH), 1200–1040 cm$^{-1}$ (ketal); $^1$H NMR ($CDCl_3$) δ 4.18–3.82 (m, $OCH_2CH_2O$), 3.25 (half of AB q, $J$ = 9.5 Hz, H-2), 3.08–2.45 (m, H-3), 2.16 (br s, $NH_2$ and OH), 2.0–1.0 (m, $3CH_2$). Anal. ($C_8H_{15}NO_3$) C, H, N.

***trans*-3-(6-Chloro-9-purinyl)-2-hydroxycyclohexanone Ethylene Ketal (5).** A solution of **4** (7.69 g, 44.4 mmol), 5-amino-4,6-dichloropyrimidine (7.28 g, 44.4 mmol), and triethylamine (19 mL, 0.13 mol) in 1-butanol (130 mL) was refluxed for 24 h. Evaporation left a brown glass which was shaken with triethyl orthoformate. The precipitate of $Et_3NH^+Cl^-$ which formed was filtered off and ethanesulfonic acid (2.0 g) added to the filtrate. After 18 h at room temperature, hexane (100 mL) was added and the resulting mixture cooled. The off-white precipitate was filtered off and crystallized from EtOAc, giving **5** as white granules (9.60 g, 70%): mp 191–193 °C dec; UV max (0.1 N HCl) 265 nm (log ε 3.99), ($H_2O$) 265 (3.99), (0.1 N NaOH) 265 (4.00); IR (KBr) 1585, 1555 cm$^{-1}$ (purine); $^1$H NMR ($Me_2SO$-$d_6$) δ 8.73 (s, $w_{1/2}$ = 2.0 Hz, purine H-2′ and H-8′), 5.02 (d, $J$ = 6.0 Hz, CHO*H*), 4.8–4.2 (m, 2CH), 4.2–3.8 (m, $OCH_2CH_2O$), 2.3–1.4 (m, $3CH_2$). Anal. ($C_{13}H_{15}ClN_4O_3$) C, H, N.

***trans*-3-(6-Dimethylamino-9-purinyl)-2-hydroxycyclohexanone Ethylene Ketal (6).** A solution of **5** (9.60 g, 30.9 mmol) in 40% aqueous $NHMe_2$ (250 mL) was refluxed for 2.0 h. The resulting mixture was filtered and the solid washed with water and dried, giving **6** as white crystals (9.08 g, 92%): mp 214–215 °C; TLC and IR identical with those of an analytical sample. Recrystallization from EtOAc–hexane gave white needles: mp 215–216 °C; $^1$H NMR ($Me_2SO$-$d_6$) δ 3.47 ($NMe_2$). Anal. ($C_{15}H_{21}N_5O_3$) C, H, N.

***trans*-3-(6-Dimethylamino-9-purinyl)-2-hydroxycyclohexanone Oxime (7).** Oximation of **6** was carried out as described previously,[1] giving **7** as a white solid (86%): mp 199.5–200.5 °C dec (from MeOH); IR (KBr) 1600, 1560, 1530 cm$^{-1}$ (oxime, purine). Anal. ($C_{13}H_{18}N_6O_2$) C, H, N.

***trans*-2-Acetoxy-3-(6-dimethylamino-9-purinyl)cyclohexanone *O*-Acetyloxime (8).** A solution of **7** (6.66 g, 22.9 mmol) in $Ac_2O$ (75 mL)–pyridine (25 mL) was maintained at 70 °C for 0.5 h. Evaporation left glass which crystallized from EtOAc–hexanes, giving **8** as white needles (7.05 g, 82%): mp 134.5–135.5 °C; IR (KBr) 1780 (NOAc), 1730 (OAc), 1600 cm$^{-1}$ (purine); $^1$H

NMR ($Me_2SO$-$d_6$) δ 8.31 and 8.27 (both s, purine H-2′ and H-8′), 6.23 (d, $J$ = 10.5 Hz, CHO), 5.3–4.5 (m, CHN), 3.50 (s, $NMe_2$), 3.3–2.4 (m, 2H-6), 1.83 and 2.21 (both s, $2CH_3C{=}O$) overlapped by 2.4–1.7 (m, $2CH_2$). Anal. ($C_{17}H_{22}N_6O_4$) C, H, N.

**Diborane Reduction of 8. Separation of 2α-Acetamido-6β-(6-dimethylamino-9-purinyl)cyclohexan-1α-ol (9) and 2α-Acetamido-6α-(6-dimethylamino-9-purinyl)cyclohexan-1β-ol (10).** The reduction was carried out exactly as for the cyclopentyl analogue.[1] Reduction of **8** (7.00 g, 18.7 mmol) gave, after acetylation, a mixture of **9** and **10** (4.85 g) which was separated by chromatography on silica gel eluted with 2–10% MeOH–$CHCl_3$. The greater $R_f$ isomer **9** was isolated as white crystals (420 mg, 7%) from EtOAc: mp 211.5–213 °C; mixture melting point with a sample prepared by alternate route (see below) was undepressed; IR (KBr) 3500–3100 ($NH_2OH$) 1663 and 1630 (amide C=O), 1600 $cm^{-1}$ (purine); IR ($CHCl_3$) 1663, 1598 $cm^{-1}$, identical with a sample prepared by an alternate route. Anal. ($C_{15}H_{22}N_6O_2$) C, H, N.

The lower $R_f$ isomer **10** was isolated as white crystals (2.02 g, 33%) from EtOAc: mp 242–243 °C; IR (KBr) 3240, 3175 sh, 3090, 3060 (OH, NH), 1648, 1560 (amide C=O), 1597 $cm^{-1}$ (purine); $^1H$ NMR ($Me_2SO$-$d_6$) δ 8.22 and 8.09 (both s, purine H-2′ and H-8′), 7.83 (d, $J$ = 6.0 Hz, NHC=O), 4.87 (d, $J$= 4.0 Hz, OH), 4.7–3.6 (m, 3CH), 3.49 (s, $NMe_2$), 1.85 (s, $CH_3C{=}O$) overlapped by 2.4–1.0 (m, $3CH_2$). Anal. ($C_{15}H_{22}N_6O_2$) C, H, N.

***cis*-3-Benzamido-1,2-epoxycyclohexane (11).** Epoxidation of 3-benzamidocyclohexene[6] (20.0 g, 99.4 mmol) was carried out with *m*-chloroperbenzoic acid by a procedure described for epoxidation of 3-acetamidocyclopentene.[3] Epoxide **11** crystallized from $CHCl_3$–hexanes as white needles (16.2 g, 75%): mp 116–117 °C (lit.[6] mp 115–116 °C).

**6β-Azido-2α-benzamidocyclohexan-1α-ol (12).** Epoxide **11** was allowed to react with $NaN_3$ using a procedure described previously,[3] giving **12** as white plates from benzene (87%): mp 117–118 °C; IR (KBr) 2080 $cm^{-1}$; $^1H$ NMR ($Me_2SO$-$d_6$) δ 8.2–7.3 (m, $C_6H_5$ and NHC=O), 5.32 (d, $J$ = 4.0 Hz, OH), 4.5–3.5 (m, 3CH), 2.3–1.0 (m, $3CH_2$). Anal. ($C_{13}H_{16}N_4O_2$) C, H, N.

**6β-Amino-2α-benzamidocyclohexan-1α-ol (13).** A solution of **12** (15.2 g, 58.4 mmol) in absolute EtOH (120 mL) was shaken with prereduced platinum oxide (500 mg) under $H_2$ (50 psi) in a Parr apparatus for 18 h. Concentration of the EtOH solution, after filtration, gave **13** as white crystals (91%): mp 168–169.5 °C; IR and $^1H$ NMR as expected. Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

**2α-Benzamido-6β-(6-dimethylamino-9-purinyl)cyclohexan-1α-ol (14).** A mixture of **13** (10.8 g, 45.8 mmol), 5-amino-4,6-dichloropyrimidine (7.25 g, 44.2 mmol), triethylamine (19 mL, 0.13 mol), and 1-butanol (150 mL) was refluxed under $N_2$ for 48 h. The resulting solution was evaporated to a yellow glass which was dissolved in $CHCl_3$ (200 mL), extracted with half-saturated $NaHCO_3$ (2 × 50 mL) and then saturated NaCl (50 mL), and dried ($CaSO_4$). Evaporation left a yellow solid foam (18.2 g) which was chromatographically homogeneous. This foam was stirred in triethyl orthoformate (250 mL) with ethanesulfonic acid (2.0 g) for 20 h. Evaporation left a brown glass which was dissolved in EtOAc, extracted with half-saturated $NaHCO_3$ and saturated NaCl, and then dried. Evaporation left a brown glass (17.8 g): NMR ($Me_2SO$-$d_6$) as expected for a mixture of the two possible ethoxyoxazolidines of the 6-chloropurine compound. Such a sample of the crude 6-chloropurine (12.0 g) was refluxed in DMF (50 mL)–40% aqueous $NHMe_2$ (200 mL) for 4.0 h. Evaporation left a brown gum which was dissolved in 2 N HCl (400 mL) and heated (steam bath) for 10 min. Evaporation and treatment of the residue with excess IRA-400 ($OH^-$) resin in MeOH gave a white solid (9.2 g). Crystallization from EtOAc gave **14** as white granules (8.9 g, 60%): mp 199–200 °C; IR (KBr) 1670, 1560 (amide), 1605 $cm^{-1}$ (purine); $^1H$ NMR ($Me_2SO$-$d_6$) δ 8.26 and 8.17 (both s, purine H-2′ and H-8′) overlapped by 8.3–7.3 (m, $C_6H_5$ and NHC=O), 5.09 (d, $J$ = 4.7 Hz, OH) overlapped by 5.4–4.2 (m, 3 CH), 3.49 (s, $NMe_2$), 2.4–1.8 (m, $3CH_2$). Anal. ($C_{20}H_{24}N_6O_2$) C, H, N.

A portion of **14** was hydrolyzed by refluxing in 0.5 N $Ba(OH)_2$ for 18 h. The resulting hygroscopic foam was shaken with $Ac_2O$ for 2 min, giving a colorless glass which crystallized from EtOAc (86%): mp 211.5–213 °C; mixture melting point with **9** undepressed; IR ($CHCl_3$) identical with that of **9** prepared as described above.

**2α-Amino-6α-(6-dimethylamino-9-purinyl)cyclohexan-1β-ol (16).** A solution of **10** (1.00 g, 2.77 mmol) in 0.5 N $Ba(OH)_2$ (50 mL) was refluxed for 3 h. EtOH (50 mL) was added and the solution neutralized with $CO_2$. The $BaCO_3$ was filtered off through Celite. The filtrate was evaporated and the residue dissolved in MeOH and passed through a column of Amberlite IRA-400 ($OH^-$) resin (10 mL) in MeOH. Evaporation of the basic MeOH eluent left a colorless glass (796 mg) which crystallized from absolute EtOH, giving **16** as white granules (592 mg, 77%): mp 207–208.5 °C; IR (KBr) 3325, 3260 ($NH_2$), 3125 (br, OH), 1595 $cm^{-1}$ (purine). Anal. ($C_{13}H_{20}N_6O$) C, H, N.

**2α-Amino-6β-(6-dimethylamino-9-purinyl)cyclohexan-1α-ol (15).** Acetamide **9** or benzamide **14** was hydrolyzed with $Ba(OH)_2$ as described above. The resulting amine **15** (96%) was a hygroscopic white solid foam which could not be crystallized and appeared to carbonate on standing, in contrast to **16** (see above). This difference between the *cis*- and *trans*-amino alcohols was also noted for the cyclopentyl analogues.[1] Freshly prepared samples were used immediately.

**9-[(*R*)-[3(*R*)-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-2(*R*)-hydroxy]cyclohexyl]-6-dimethylaminopurine and 9-[(*S*)-[3(*S*)-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-2(*S*)-hydroxy]cyclohexyl]-6-dimethylaminopurine (17a and 17b).** A freshly prepared sample of racemic amine **15** was condensed with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamine[11] by the *N*-hydroxysuccinimide–DCC method, as described previously.[1] The resulting mixture of diastereomers, **17a** and **17b**, was a chromatographically homogeneous white solid foam (97%) suitable in purity for the next reaction. An analytical sample of the mixture was prepared by chromatography on preparative silica gel plates developed in 10% MeOH–$CHCl_3$, giving a white solid foam: IR (KBr) 3550–3150 (br, OH, NH), 1700 (Cbz), 1655, 1545 $cm^{-1}$ (br, amide). Anal. ($C_{31}H_{37}N_7O_5$) C, H, N.

**9-[(*R*)-[3(*S*)-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-2(*R*)-hydroxyl]cyclohexyl]-6-dimethylaminopurine and 9-[(*S*)-[3(*R*)-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-2(*S*)-hydroxy]cyclohexyl]-6-dimethylaminopurine (18a and 18b).** Racemic amine **16** was condensed with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamine[11] as above. The resulting mixture of diastereomers, **18a** and **18b**, was a colorless glass (93%) which was chromatographically homogeneous. An analytical sample of the mixture was prepared by solidification from EtOAc, giving white powder (69%): mp 188–192 °C; IR (KBr) 3400, 3200 (OH, NH), 1695 (Cbz), 1648, 1560, 1535 $cm^{-1}$ (amide). Anal. ($C_{31}H_{37}N_7O_5$) C, H, N.

**6-Dimethylamino-9-[(*R*)-[2(*R*)-hydroxy-3(*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (1a) and 6-Dimethyamino-9-[(*S*)-[2(*S*)-hydroxy-3(*S*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (1b).** A mixture of **17a** and **17b** (4.00 g, 6.81 mmol) was dissolved in glacial AcOH (200 mL) and shaken with 10% Pd/C (2.0 g) under $H_2$ (10 psi) in a Parr apparatus for 1.0 h. The catalyst was filtered off and the AcOH evaporated. The residue was dissolved in MeOH and passed through a column of Amberlite IRA-400 ($OH^-$) resin (50 mL). The MeOH eluent was evaporated to dryness, leaving a mixture of **1a** and **1b** as a white solid foam (2.78 g, 90%); TLC (15% MeOH–$CHCl_3$) shows two bands at lower $R_f$ than starting material; IR and $^1H$ NMR as expected; attempts to crystallize the mixture were unsuccessful. The diasteromers were separated by preparative TLC (20% MeOH–$CHCl_3$), giving white solid foams. The greater $R_f$ diastereomer was assigned structure **1b** (see discussion): $[\alpha]^{25}_{589}$ +21.1°, $[\alpha]^{25}_{436}$ +42.9° (*c* 0.25, $CHCl_3$); IR (KBr) 3300 (br, OH, NH, $NH_2$), 1658 (amide), 1597 $cm^{-1}$ (purine); $^1H$ NMR ($CDCl_3$) σ 8.13 and 7.73 (both s, H-8 and H-2), overlapping 7.8 (br, NH), 6.88 (q, $J$ = 8 Hz, $MeOC_6H_4$), remainder of spectrum showed overlapping with discernible singlets at 3.55 (OMe) and 3.40 ($NMe_2$); mass spectrum (probe temperature 200 °C, 70 eV) *m/e* (rel intensity) 453 (3.3, $M^+$), 436 (1.3), 435 (2.3, M – 18), 418 (2.7), 368 (2.8), 333 (7.6), 332 (36.5, M – $CH_2C_6H_4OMe$), 315 (19.6), 314 (62.2, M – $CH_2C_6H_4OMe$ – $H_2O$), 304 (12.0), 286 (7.0), 285 (6.3), 261 (12.4), 256 (5.1), 244 (8.1), 218 (4.0, B + $C_4H_8$), 205 (4.2, B + $C_3H_7$), 190 (3.5, B + $C_2H_4$), 165 (11.6), 164 (100, B + 2H), 163 (39.1, B + H), 150 (17.1, $NH_2CHCH_2C_6H_4OMe$), 134 (22.8, B + H – $CH_3N$), 121 (23.9,

$CH_2C_6H_4OMe$), 96 (21.8), 83 (19.5). Anal. ($C_{23}H_{31}N_7O_3$) C, H, N.

The lower $R_f$ diastereomer was assigned structure **1a** (see discussion): $[\alpha]^{25}_{589}$ −99.7°, $[\alpha]^{25}_{436}$ −223.6° (*c* 0.23, $CHCl_3$); IR, $^1H$ NMR, and mass spectra almost identical with those of 1b. Anal. ($C_{23}H_{31}N_7O_3$) C, H, N.

**6-Dimethylamino-9-[(*R*)-[2(*R*)-hydroxy-3(*S*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine and 6-Dimethylamino-9-[(*S*)-[2(*S*)-hydroxy-3(*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclohexyl]purine (2a and 2b).** Hydrogenolysis of a mixture of **18a** and **18b** as described above gave a mixture of **2a** and **2b** as a colorless glass (90%): TLC (10% MeOH–$CHCl_3$) shows two bands at lower $R_f$ than starting material; IR and $^1H$ NMR as expected; attempts to crystallize the mixture were unsuccessful. The diastereomers were separated by preparative TLC (20% MeOH–$CHCl_3$), giving hygroscopic glasses. The greater $R_f$ diastereomer was designated **2b**: $[\alpha]^{25}_{589}$ −78.0°, $[\alpha]^{25}_{436}$ −175.5° (*c* 0.23, $CHCl_3$); IR (KBr) 3300 (br, OH, NH, $NH_2$), 1655 (amide), 1595 $cm^{-1}$ (purine); $^1H$ NMR ($CDCl_3$) δ 8.07 and 7.64 (both s, H-8 and H-2), 7.33 (br, NH), 6.83 (q, $J$ = 8 Hz, $MeOC_6H_4$), remainder of spectrum showed overlapping with discernible singlets at 3.72 (OMe) and 3.38 ($NMe_2$); mass spectrum (probe temperature 200 °C, 70 eV) *m/e* (rel intensity) 453 (8.6, $M^+$), 437 (1.6), 436 (1.6), 435 (1.2), 419 (0.9), 368 (5.4), 333 (17.9), 332 (92.7, M − $CH_2C_6H_4OMe$), 315 (3.2), 314 (3.7, M − $CH_2C_6H_4OMe$ − $H_2O$), 304 (8.6), 303 (9.5), 285 (3.5), 276 (14.6), 261 (5.8), 256 (9.7), 244 (5.2), 218 (6.9, B + $C_4H_8$), 205 (10.0, B + $C_3H_7$), 190 (3.8, B + $C_2H_4$), 165 (14.5), 164 (100, B + 2H), 163 (25.7, B + H), 150 (21.5, $NH_2CHCH_2C_6H_4OMe$), 134 (17.8, B + H − $CH_3N$), 121 (25.7, $CH_2C_6H_4OMe$), 97 (40.7), 83 (59.2). Anal. ($C_{23}H_{31}N_7O_3 \cdot 0.5H_2O$) C, H, N.

The lower $R_f$ diastereomer was designated **2a**: $[\alpha]^{25}_{589}$ −5.02°; $[\alpha]^{25}_{436}$ −7.53° (*c* 0.24, $CHCl_3$); IR, $^1H$ NMR and mass spectra almost identical with those of **2b**. Anal. ($C_{23}H_{31}N_7O_3 \cdot H_2O$) C, H, N.

**6-Dimethylamino-9[(*R*)-[2(*R*)-hydroxy-3(*S*)-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl]purine and 6-Dimethylamino-9-[(*S*)-[2(*S*)-hydroxy-3(*R*)-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl]purine (19a and 19b).** Hydrolysis of 2α-acetamido-5α-(6-dimethylamino-9-purinyl)-cyclopentan-1β-ol[1] with refluxing 0.5 N $Ba(OH)_2$ gave a quantitative yield of amine which was immediately coupled with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamine[11] as above. The usual work-up gave a white solid foam (84%): chromatographically homogeneous; IR (KBr) almost identical with that of the *N*-benzyloxycarbonyl derivative of **20a** or **20b**.[1] Hydrogenolysis as above gave a mixture of diastereomers **19a** and **19b** (90%), which were separated by preparative TLC (15% MeOH–$CHCl_3$). The greater $R_f$ diastereomer **19b** was isolated as a solid foam: $[\alpha]^{25}_{589}$ −61.6°, $[\alpha]^{25}_{436}$ −143.7° (*c* 0.23, $CHCl_3$); IR (KBr) 3300 (br, OH, NH, $NH_2$), 1650 (amide), 1600 $cm^{-1}$ (purine); mass spectrum (probe temperature 200 °C, 70 eV) *m/e* (rel intensity) 439 (2.4, $M^+$), 422 (0.8), 421 (1.0), 319 (22.3), 318 (100, M − $CH_2C_6H_4OMe$), 301 (0.6), 300 (1.5, M − $CH_2C_6H_4OMe$ − $H_2O$), 273 (1.5), 230 (4.4), 190 (14.4, B + $C_2H_4$), 164 (95.2, B + 2H), 163 (16.9, B + H), 150 (14.7, $NH_2CHCH_2C_6H_4OMe$), 148 (11.1), 134 (23.4, B + H − $CH_3N$), 122 (7.8), 121 (33.9, $CH_2C_6H_4OMe$), 120 (6.4), 109 (2.0), 108 (2.4), 82 (5.0). Anal. ($C_{22}H_{29}N_7O_3$) C, H, N.

The lower $R_f$ diastereomer **19a** was also a solid foam: $[\alpha]^{25}_{589}$ −25.8°, $[\alpha]^{25}_{436}$ −61.4° (*c* 0.23, $CHCl_3$); IR (KBr) and mass spectra almost identical with those of **19b**. Anal. ($C_{22}H_{29}N_7O_3$) C, H, N.

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# Isoxazole Anthelmintics

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A series of 3-halo-5-phenyl- and 3-phenyl-5-haloisoxazoles has demonstrated anthelmintic activity at doses ranging from 16 to 500 mg/kg orally against the rat roundworm, *Nippostrongylus braziliensis*. In the 5-phenyl series a halogen at the 3 position of the isoxazole ring was required for activity. However, in the 3-phenyl series activity was maintained after replacement of the 5-halogen with certain alkoxyl, thioalkoxyl, or amino groups. The 3-phenyl and 5-phenyl series apparently are not acting biologically at a common receptor site. Synthetic methods and structure–activity relationships are discussed.

During the testing of selected compounds for anthelmintic activity in a rodent parasite screen, 3-chloro-5-phenylisoxazole (1) exhibited anthelmintic activity against the rat roundworm, *Nippostrongylus braziliensis*, equal to that of the commericial anthelmintic 2-(4-thiazolyl)-benzimidazole (thiabendazole). Although 1 is a known compound,[1] no report of anthelmintic activity for 1 or other 5-phenylisoxazoles was found.

Although the literature is replete with references to the biological activity of compounds containing the isoxazole ring as part of their structures, only in five instances were claims of anthelmintic activity made for such compounds. Sen and co-workers[2] claim cestocidal activity for a series of 3-aryl-5-(halomethyl)isoxazoles. A series of 5-amino- and 5-acylamino-3-pyridylisoxazoles has been claimed as anthelmintics and nematocides.[3,4] The corresponding 3-aryl-5-aminoisoxazoles also have been claimed as antiparasitics.[5] Anthelmintic, coccidiostatic, and growth-promoting activity has been claimed for a class of 5-amino-4-(5-nitro-2-furyl)-4-(substituted carbonyl)isoxazoles.[6]

The lack of a study of 5-phenylisoxazoles as anthelmintics prompted our investigation of this class of compounds. During the course of this work, the isomeric