concns of inhibitor. The *in vitro* antitumor assays were carried out by our microassay technique which involves the introduction of 0.5 ml aliquots of the medium (RPMI 1630 + 10% calf serum) contg the various concns of the analog into 16 × 125 mm screw cap culture tubes, followed by 0.5 ml portions of medium contg 3×10^5 L-1210 cells. The cultures are incubated at 37° for 40 hr, after which the viable cells are counted by trypan blue exclusion. During this time the cell number in the controls increases approximately eight- to ninefold, with an average viability of 99%.

Acknowledgment. The excellent technical assistance of Miss Ginger Dutschman and Mr. R. J. Maue is gratefully acknowledged. This study was aided by grants CA-12585 and 12422 from the U. S. Public Health Service and T-436 from the American Cancer Society.

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Synthesis and Antimicrobial Activity of a Carbocyclic Puromycin Analog. 6-Dimethylamino-9-{*R*-[2*R*-hydroxy-3*R*-(*p*-methoxyphenyl-L-alanylamino)]-cyclopentyl}purine†

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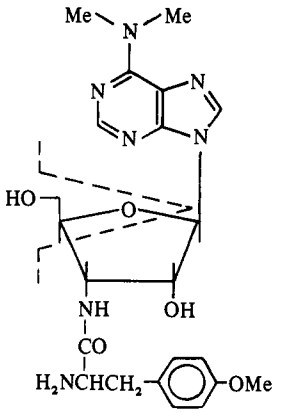
An assessment of the requirement for the furanosyl O and the CH_2OH moiety in the puromycin molecule was undertaken by the synthesis of a novel puromycin analog. A carbocyclic analog, 6-dimethylamino-9-{*R*-[2*R*-hydroxy-3*R*-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl}purine (**2**), was synthesized and evaluated for antimicrobial activity. The carbocyclic analog exhibited antimicrobial activity comparable to puromycin, and also circumvented the nephrotic syndrome associated with puromycin by releasing a nontoxic aminonucleoside upon hydrolysis. The diastereoisomer (**19**) of **2** was also isolated and found to be devoid of antimicrobial activity.

Puromycin (**1**), an antibiotic with antitumor activity,[1] has been found to inhibit protein synthesis in a wide variety of organisms. Its structure has a striking resemblance to that of the aminoacyl-adenyl terminus of aminoacyl-tRNA, and it has been demonstrated that the antibiotic causes premature release of the polypeptide chains from the ribosome.[2] For this reason, puromycin has been used extensively as a tool in the investigation of protein biosynthesis.



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A variety of analogs and isomers of puromycin have been prepared to define the structural requirements for inhibition in an attempt to further understand its mode of action.[3,4] However, all of these structures have been of the classical nucleoside type in which an N-substituted amino sugar is attached to a purine or pyrimidine ring through a glycosidic linkage.[3-6] The difficulties encountered in preparing 3-aminoribosyl nucleosides have severely limited the availability of these compounds. Also, the classical nucleoside compounds introduce two undesirable structural features into the puromycin analogs which have not been demonstrated as essential for biological activity; *i.e.*, the furanosyl O and the 5′-OH group. Thus, it may be possible to modify **1** within the region outlined by the dotted line and still retain the activity of the antibiotic.

Since ribonucleosides are easily cleaved hydrolytically or enzymatically, many nucleosides which may be effective chemotherapeutic agents become ineffective *in vivo* because they are rapidly destroyed by cleavage into a purine or pyrimidine and a carbohydrate moiety.[7,8] This difficulty could be circumvented by replacing the furanosyl ring with a cyclopentyl system which sterically simulates the sugar moiety and provides a hydrolytically stable C-N bond. The removal of the 5′-OH group from puromycin and its analogs would be desirable from a toxicity standpoint. Toxic manifestations, including renal lesions, have precluded the use of puromycin in the treatment of human or animal infectious diseases or neoplasms.[9] The nephrotic syndrome results from small amounts of aminonucleoside produced by the hydrolytic removal of the amino acid moiety from administered puromycin.[9] Recent studies demonstrate that the aminonucleoside is first monodemethylated[10] and subsequently converted to the 5′-nucleotide.[11] It has been sug-

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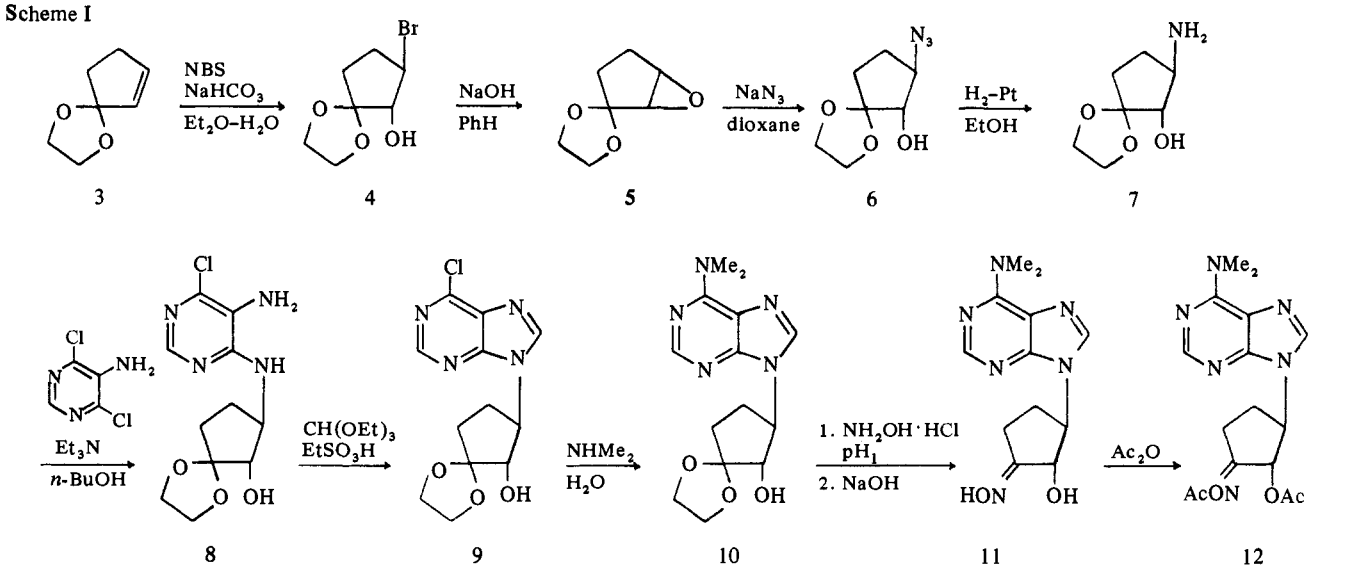
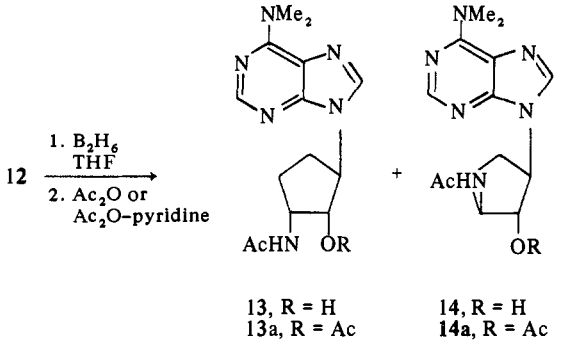
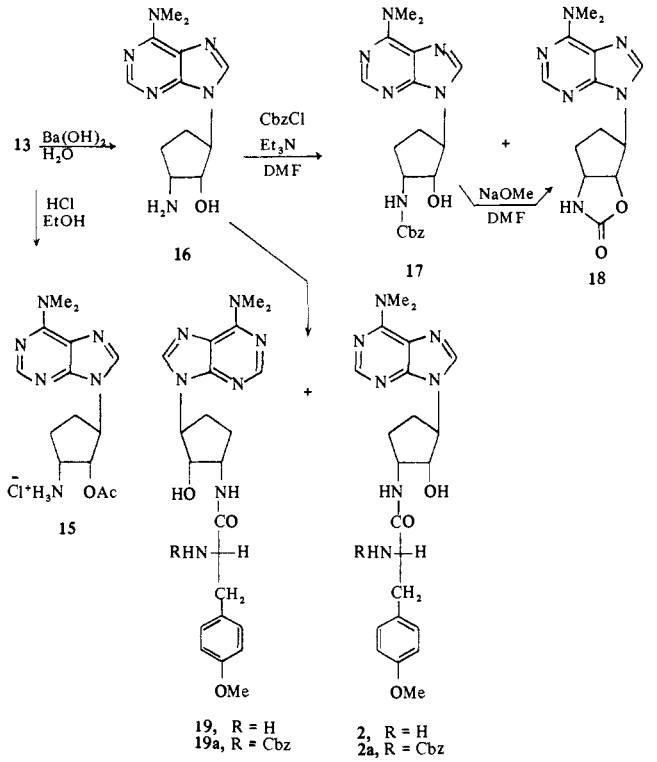
Scheme I







gested that this 5′-nucleotide is responsible for the nephrotic syndrome.[11]

In view of these observations, we decided to synthesize the carbocyclic analog **2** which retains the structural requirements that have thus far been demonstrated to be essential for activity.[3] The requirement for the 2′-OH in puromycin has not been demonstrated. However, it is required for the acceptor activity of aminoacyl adenosines[12] which are thought to bind at the ribosomal site in the same manner as puromycin. Thus, the antimicrobial activity of the carbocyclic analog, **2**, would provide information relating to the structural requirements of the 4′ and 5′ oxygens of puromycin for biological activity. In addition, **2** represents a new class of puromycin analogs which should produce a nontoxic aminonucleoside upon hydrolysis of the amino acid moiety.

Chemistry. The carbocyclic analog (**2**) of puromycin was synthesized by the route shown in Scheme I. (Structures **3**–**18** depict only one enantiomer of the racemic form actually obtained.) Cyclopent-2-enone ethylene ketal (**3**)[13] was treated with NBS in Et_2O-H_2O by a modification of the procedure of Guss and Rosenthal[14] in which $NaHCO_3$ was used to buffer the reaction mixture. The resulting bromohydrin **4** decomposed on storage and was not characterized.‡ When bromohydrin **4** was immediately treated with NaOH in refluxing benzene, epoxide **5** was formed in 73% yield (from **3**), as a stable colorless liquid. The epoxide was opened with NaN_3 in dioxane by the method of Vander Werf, *et al.*,[15] and the resulting azide reduced catalytically to crystalline *trans*-3-amino-2-hydroxycyclopentanone ethylene ketal (**7**) in 76% yield (from **5**). Attack by N_3^- at C-3 of epoxide **5** would be expected.[16] The structure of **7** was confirmed by the nmr spectrum in which H-2 appears as a doublet at τ 6.53 (J = 7.9 Hz), and H-3 appears upfield as a multiplet at τ 7.3-6.8.

The purine moiety was formed *via* a standard method.[17] Condensation of **7** with 5-amino-4,6-dichloropyrimidine, followed by ring-closure of the resulting crude pyrimidine **8** with triethyl orthoformate in the presence of $EtSO_3H$ gave the 6-chloropurine **9** in 84% yield (from **7**). The 6-dimethylaminopurine **10** was formed in 75% yield when **9** was treated with refluxing Me_2NH.

The ketal function of **10** proved unusually resistant to hydrolysis. Attempted hydrolysis by refluxing HCl at pH 3, refluxing aq NH_4Cl, or transketalization in acetone with *p*-TsOH gave only recovered **10**. This difficulty has been encountered in the hydrolysis of other ketals and acetals of similar purine derivatives.§ The proximity of a protonated

‡ The corresponding bromohydrin of cyclohex-2-enone ethylene ketal prepd by this method was a stable, cryst solid, mp 96-98°, which was characterized; unpublished results.

§ Unpublished results.

amine has been found to hinder acetal hydrolysis.[18] The neighboring OH would not be expected to affect the rate of hydrolysis of the ketal significantly.[19] It thus seems likely that the protonated purine moiety accounts for the difficulty of hydrolysis of compounds such as **10**.

When **10** was refluxed in HCl at pH 1, black tar formed on neutralization. Similarly, treatment with Amberlite IR-120 resin at 60° for 2 hr led only to decomposition. As it appeared that conditions necessary for hydrolysis of **10** effected decomposition of the product(s), oxime **11** was synthesized directly from **10** under what appear to be unique conditions for oxime formation. An aq soln of **10** and a 5-fold excess of $HONH_2 \cdot HCl$ adjusted to pH 1 with HCl was warmed (75°) for 3 hr. On neutralization of the colorless reaction solution, pure **11** precipitated immediately in almost quantitative yield (solid forms as pH 3.5 is reached). However, when oxime formation was attempted by warming a solution of **10** at pH 1, and *then* adding $HONH_2 \cdot HCl$ along with sufficient NaOH to give a pH of 6, partial recovery of **10** (69%) and considerable darkening of the reaction solution resulted. Even in the presence of $HONH_2 \cdot HCl$, dark tar and a lowered yield of oxime (43%) were obtained if a pH slightly lower than 1 was used. Identical conditions at a pH of 3 gave only recovered **10**. These results suggest that hydrolysis of the ketal of **10** requires a pH lower than 3, but that below pH 1, **10** or its hydrolysis products is unstable and decomposes rapidly before hydroxylamine may attack. In a rather narrow pH range, $HONH_2$ appears to prevent decomposition, probably by direct attack on the resonance-stabilized carbonium ion formed on ring opening of the protonated ketal.

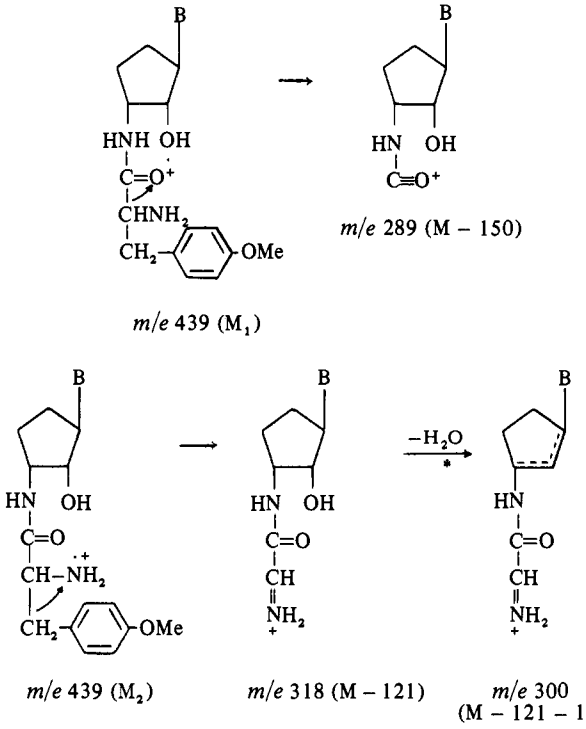
Tlc of **11** indicated it to be a mixture of syn and anti oximes. Since attempted separation by chromatography or recrystallization resulted in considerable losses, the mixture was acetylated without separation, giving a 70% yield of a chromatographically homogeneous diacetyl derivative (**12**). The nmr spectra of **11** and **12** confirm the structures shown. In particular, the appearance of H-2 as a doublet ($J_{2,3}$ = 9.0 Hz for **11** and 9.5 Hz for **12**) considerably downfield from the H-3 multiplet# indicates that the oximation conditions did not result in isomerization *via* an enediol.

The *O*-acetyl oxime **12** was reduced to a mixture of amino alcohols with diborane in THF by a modification of the procedure used by Feuer and Braunstein for the conversion of cyclohexanone *O*-acetyloxime to cyclohexylamine.[20] This mixture was acetylated in Ac_2O and the resulting acetamides, **13** (45%) and **14** (4%), were separated by chromatography and characterized. The AcNH and OH groups were shown to be cis in **13** and trans in **14** by acyl migration studies (see Experimental Section). The diacetyl derivatives **13a** (39%) and **14a** (3%) were isolated when the mixture of amino alcohols from reduction of **12** was treated with Ac_2O-pyridine, and **13a** was converted to **13** on treatment with NH_3 in MeOH. Hydrolysis of **13** with $Ba(OH)_2$ gave a carbocyclic analog of the puromycin aminonucleoside, characterized as its AcOH salt **16**. The cis stereochemistry of the H_2N and OH groups was further confirmed by facile formation of the cyclic carbamate **18** on treatment of the carbobenzoxy derivative **17** with NaOMe in DMF.

The diborane reduction of **10** appears to be the first reported example of the reduction of an oxime to an amine in the presence of a purine ring. Predominant attack of hydride from the purine side of the cyclopentyl ring, if general, could be useful in the synthesis of amino sugar nucleosides. It is hoped that further studies now in progress on the diborane reduction of analogs of **10** will provide information on the mechanism of this highly stereospecific reduction.**

The carbocyclic aminonucleoside analog **16** was coupled to *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[4] by 2 methods: A, the dicyclohexylcarbodiimide-*N*-hydroxysuccinimide method[21,22] and B, a modification of the mixed anhydride method suggested by Anderson, *et al.*[23] The resulting carbobenzoxy blocked diastereomers **2a** and **19a** (97% by method A, 77% by method B) could not be separated. The mixture of amino alcohols from the reduction of **12** was also coupled to *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine by method A, giving a yield of **2a** and **19a**, after chromatography, comparable with the overall yield *via* **13** and **16**. Following hydrogenolysis of the Cbz group, separation of diastereomers **2** and **19** by chromatography was possible. Structure **2** is assigned to the diastereomer having $[\alpha]^{25}D$ of −83° and structure **19** to the diastereomer having $[\alpha]^{25}D$ of −8° (see Results and Discussion). The two coupling methods resulted in samples of **2** and **19** with identical optical purities.

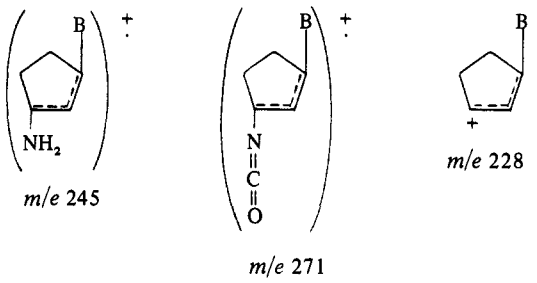
The mass spectra of **2** and **19** are almost identical, differing slightly only in the relative intensities of some ions. The molecular ion (*m/e* 439) is relatively small, and there is a minor (M − 18) peak due to loss of H_2O involving the OH group. As with puromycin,[24] the fragmentation is dominated by the aminoacyl moiety. Fission of the bond α to the C=O



in the molecular ion M_1 accounts for the *m/e* 289 (M − 150) peak. Cleavage of the benzylic bond followed by loss of H_2O accounts for the base peak at *m/e* 300 (M − 121 − 18) and prominent peaks at *m/e* 318 (M − 121) and *m/e* 121. There is a metastable peak for the transition $318^+ \rightarrow 300^+ + 18$. The (M − 121 − 18) ion is somewhat less abundant for puromycin. This would be expected as the puromycin (M − 121

#In the nmr (DMSO-d_6) of *trans*-2-acetoxy-3-(6-dimethylamino-9-purinyl)cyclohexanone *O*-acetyloxime, the cyclohexyl analog of **12**, H-2 also appears as a doublet (τ, 3.77, $J_{2,3}$ = 10.5 Hz) downfield from the H-3 multiplet at τ 5.1; unpublished results.

**Further details concerning this reaction will be published.



– 18) ion has several favorable routes for further fragmentation which these carbocyclic analogs lack, *e.g.*, loss of the elements of CH_2O involving the 5′-OH. Fission of the *m/e* 300 ion on either side of the amide carbonyl accompanied by proton transfers accounts for the *m/e* 245 and 271 peaks. Fission of the bond between the aminoacyl group and the cyclopentyl ring accounts for the *m/e* 228 ion. There is a metastable peak for the transition $300^+ \rightarrow 228^+ + 72$. As with puromycin, the *m/e* 300 peak further fragments to give a prominent *m/e* 164 (B + 2H) peak characteristic of the dimethylaminopurine moiety, as indicated by an appropriate metastable peak. Other fragmentations directed by the purine moiety account for ions of *m/e* 206 (B + 44), 190 (B + 28), 163 (B + H), 162 (B), and 134 (B + H – CH_3N). These ions, or corresponding ones, are prominent in the spectra of adenosines[25] and are also abundant in the spectrum of puromycin. It is consistent with the structure proposed for the (B + 30) ion in adenosines, which incorporates the ribose ether oxygen,[25] that replacement of O by CH_2 results in a shift of 2 mass units to (B + 28).

Results and Discussion

Antimicrobial testing of diastereomers **2** and **19** revealed that 1 isomer was completely inactive while the other exhibited growth inhibition on the same order of magnitude as puromycin. The absolute stereochemistry of the active isomer has tentatively been assigned that of structure **2** on the basis of its biological activity and in accordance with the stereochemistry of puromycin. The minimum inhibitory concns by a 2-fold serial dilution test in broth for puromycin and the carbocyclic analog **2**, respectively, are as follows (m*M*): *Staphylococcus aureus* (NRRL B-313), 0.244 and 0.244; *Bacillus subtilis* (NRRL B-545), 0.030 and 0.060; *Klebsiella pneumoniae* (NRRL B-117), 0.485 and 0.485; *Escherichia coli* (NRRL B-210) 0.060 and 0.120. A growth curve for *S. aureus* in the presence of different concns of puromycin or the carbocyclic compound is illustrated in Figure 1. A lag period is observed with both compounds when the concns are lower than those required for complete inhibition. Such a lag period is consistent with the mechanism of action of puromycin[2] since the antibiotic would be expected to be consumed as it is incorporated into the growing peptide chains.

The aminonucleoside **16** was tested for nephrotoxicity in rats at a dose of 33 mg/kg under the same conditions that are required for puromycin aminonucleoside to cause severe nephrotic syndrome at 15 mg/kg.[26] No nephrotoxicity was observed even after 17 days of treatment with **16**.

The novel puromycin analog **2** provides a molecule with the structural features required for puromycin-like antimicrobial activity. Thus, the ribofuranosyl ring can be replaced with the more hydrolytically stable cyclopentane ring without loss of activity. In addition, the removal of the CH_2OH moiety is not detrimental to activity and at the same time provides **2** with a resistance to kinase activity upon release of the aminonucleoside. This resistance to phosphorylation circumvents the nephrotic syndrome associated with puromycin aminonucleoside. This carbocyclic analog and others which are under preparation are being evaluated for *in vitro* inhibition of protein biosynthesis in an attempt to explore the requirements for binding to ribosomes. Preliminary studies with **2** and **19** on an *E. coli* ribosomal system are consistent with the antimicrobial activities. The details of these experiments will be the subject of a future paper.

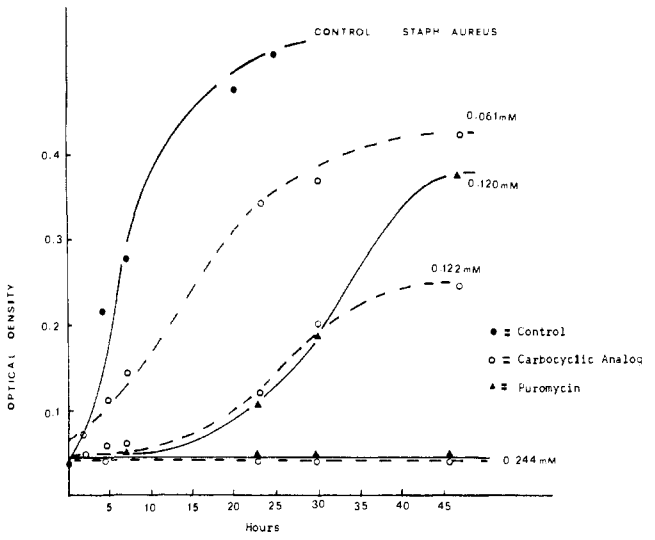


Figure 1. Growth curves of *S. aureus* in the presence of puromycin and the carbocyclic analog (**2**). Tubes containing 2-fold dilutions of the test compound in nutrient broth were inoculated with *S. aureus* and growth was measured by increase in absorption at 650 mμ.

Experimental Section††

6-Oxabicyclo[3.1.0]hexan-2-one Ethylene Ketal (**5**). The method of prepn of **4** is a modification of that of Guss and Rosenthal.[14] Cyclopent-2-enone ethylene ketal (**3**)[13] (37.85 g, 0.300 mole), NBS (53.40 g, 0.300 mole), $NaHCO_3$ (4.20 g, 50.0 mmoles), Et_2O (240 ml), and H_2O (240 ml) were stirred vigorously for 6.5 hr, at which time all of the solid had disappeared and the pH was approx 7. The aq layer was satd with NaCl, and the Et_2O layer then sepd. The aq layer was extd with addnl Et_2O (2 × 100 ml). The combined Et_2O layers were washed with satd NaCl and dried ($CaSO_4$). Evapn left crude bromohydrin **4** as a yellow oil (70 g). In another run, this oil was partially solidified from petr ether (bp 30–60°), giving gummy white crystals: mp 42–47°; ir (Nujol) 3450 (OH), 1200–1040 cm^{-1} (CO). This solid could not be recrystd and darkened on standing.‡ Best overall yields of epoxide were obtd by using the crude oil immediately. Crude **4** was dissolved in PhH (600 ml) and refluxed with powd NaOH (36 g) for 1.0 hr. The mixt was filtered, and the black solid washed with addnl PhH (200 ml). Evapn of the combined PhH soln and wash left a pale yellow liq which was distd, giving a forerun which ir showed to be a mixt of **3** and unketalized material (1.36 g),

††Melting points were detd on a Mel-Temp apparatus and are corrected. Optical rotations were measured at ambient temps with a Perkin-Elmer 141 automatic polarimeter; nmr, with a Varian A-60D spectrometer; ir, with a Perkin-Elmer 237B spectrophotometer; uv, with a Cary 14 recording spectrophotometer; low-resolution, 50-eV mass spectra, with a Hitachi Perkin-Elmer RMU-6D mass spectrometer (ion source temperature 250°, accelerating potential 1800V), equipped with a direct inlet probe. Tlc was run on silica gel (Eastman chromagram sheets with fluorescent indicator) in these solvent systems: A, 2% MeOH–$CHCl_3$; B, 5% MeOH–$CHCl_3$; C, 10% MeOH–$CHCl_3$; D, 15% MeOH–$CHCl_3$; E, 20% MeOH–$CHCl_3$. Prep tlc was done on 20 × 20 cm glass plates coated with 2 mm of silica gel F 254 (E. Merck, Darmstadt) and column chromatog on silica gel (Baker, AR, 60–200 mesh). Evapns were carried out *in vacuo* with a bath temp of less than 45° unless otherwise noted. Solid samples were dried *in vacuo* (<1 mm) at 56° before analysis. Analytical results are within ±0.4% of the calcd values. Celite is a diatomaceous earth product of Johns-Manville.

bp 28–86° (7 mm), followed by **5** (31.28 g, 73%), bp 86.5–88° (7 mm); ir identical with that of the analytical sample. Redistn of such a sample gave the analytical sample of **5** as a colorless liq, bp 98–99° (10 mm), n^{20}D 1.4698; ir and nmr were as expected. *Anal.* ($C_7H_{10}O_3$) C, H.

Samples of **5** (distd once) were stored for months at 5° without deterioration.

***trans*-3-Amino-2-hydroxycyclopentanone Ethylene Ketal (7).** The method of prepn of **6** is that of Vander Werf, *et al.*[15] A soln of **5** (2.84 g, 20.0 mmoles) in dioxane (40 ml) was brought to reflux. A soln of NaN_3 (1.63 g, 25.0 mmoles) in H_2O (10 ml) was added to the refluxing soln over 1.0 hr. The resulting mixt was refluxed with vigorous stirring for 48 hr. The dioxane layer was sepd from the aq layer, and the aq layer was extd with addnl dioxane (50 ml). The combined dioxane layers were washed with satd NaCl (50 ml) and dried ($CaSO_4$). Evapn (25°, 0.5 mm) left **6** as a pale yellow gummy solid (3.46 g); ir (Nujol) 3300 (OH), 2093 cm^{-1} (N_3). This material was dissolved in abs EtOH (75 ml) and shaken with PtO_2 (100 mg) under H_2 (50 psi) for 20 hr. After filtration, the EtOH was evapd, leaving pale yellow solid (2.91 g) which ir showed to contain no azide. Crystn from PhH gave 7 as pale yellow crystals (2.41 g, 76%); mp 97–98°; ir identical with that of the analytical sample. Sublimation of such a sample at 90–100° (0.20 mm) gave the analytical sample of 7 as white needles: mp 99–100°; ir as expected; nmr ($CDCl_3$) τ 8.9–7.8 (m, 4, 2 CH_2), 7.68 (s, 3, NH_2 and OH), 7.3–6.8 (m, 1, H-3), 6.53 (d, 1, $J_{2,3}$ = 7.9 Hz, H-2), 6.1–5.9 (m, 4, ethylene ketal). The singlet at τ 7.68 disappeared on addn of D_2O. *Anal.* ($C_7H_{13}NO_3$) C, H, N.

When run on a larger scale, the yield of 7 was lower, *e.g.*, 0.18 mole of **5** gave a 54% yield of 7 (after crystn from PhH).

***trans*-3-(6-Chloro-9-purinyl)-2-hydroxycyclopentanone Ethylene Ketal (9).** A soln of 7 (14.04 g, 88.20 mmoles), 5-amino-4,6-dichloropyrimidine (14.47 g, 88.20 mmoles), and Et_3N (37 ml, 265 mmoles) in *n*-BuOH (160 ml) was refluxed under N_2 for 44 hr. Evapn (50°, 0.2 mm) left a brown oil (42 g) contg **8** which was shaken with triethyl orthoformate (200 ml) for a few min. The white solid $Et_3NH^+Cl^-$ (10.82 g, 89%) did not dissolve and was removed by filtration. $EtSO_3H$ (1.0 g) was added to the filtrate, and it was stirred at ambient temp for 15 hr. At this time solid had formed. Hexane (200 ml) was added, and the mixt was cooled. The tan solid was collected and washed with hexane (100 ml), giving crude **9** (24.42 g). Crystn from EtOAc (500 ml) gave tan needles (22.03 g, 84% from 7), mp 165–167°; ir identical with that of the analytical sample. Such a sample was recrystd twice from EtOAc, giving the analytical sample of 9 as white needles, mp 164.5–166.5°; R_f 0.50 in solvent B;†† ir, uv, and nmr as expected. *Anal.* ($C_{12}H_{13}N_4O_3Cl$) C, H, N.

***trans*-3-(6-Dimethylamino-9-purinyl)-2-hydroxycyclopentanone Ethylene Ketal (10).** A soln of 9 (3.85 g, 13.0 mmoles) in aq 25% Me_2NH (100 ml) was refluxed 3.0 hr. The soln was concd to 20 ml and extd with EtOAc (3 × 100 ml). The EtOAc ext was dried ($CaSO_4$) and concd to 50 ml. White crystals of **10** were collected (2.99 g, 75%), mp 156–158°; ir identical with that of the analytical sample. Recrystn of such a sample from EtOAc gave the analytical sample of 10 as white crystals, mp 156–158°; R_f 0.54 in solvent B; uv, and ir as expected; nmr (DMSO-d_6) τ 6.54 (s, 6, $N(CH_3)_2$). *Anal.* ($C_{14}H_{19}N_5O_3$) C, H, N.

***trans*-3-(6-Dimethylamino-9-purinyl)-2-hydroxycyclopentanone Oxime (11).** To a stirred mixt of **10** (10.00 g, 32.8 mmoles), $NH_2OH \cdot HCl$ (11.38 g, 164 mequiv), and H_2O (140 ml) was added 2 *N* HCl (approx 28 ml) dropwise until the pH was 1.0 (measured on a meter), and all solid had dissolved. The soln was stirred at 70–75° for 3.0 hr. The pH of the hot soln was then adjusted to 6.5 with 6 *N* NaOH, and white solid began to ppt. The mixt was cooled (5°) for several hours. The solid was collected, washed with H_2O (50 ml), and air-dried, giving 11 (8.95 g, 99%), mp 202–204° dec; tlc indicates a mixt of syn and anti oximes, R_f 0.47 and 0.39 in solvent C; ir differs slightly in the fingerprint region from the analytical sample; nmr identical. Such samples of 11 were sufficiently pure for use. The analytical sample of 11 was prepd by crystn from EtOAc–hexane, giving white needles, mp 208–209° dec; tlc still shows 2 spots, with somewhat different relative intensities; ir as expected; nmr (DMSO-d_6) τ 8.0–7.3 (m, overlaps DMSO-d_5, $2CH_2$), 6.52 (s, 6, $N(CH_3)_2$), 5.7–5.1 (m, 1, H-3), 5.1–4.8 (m, 1, H-2), 4.3 (m, 1, OH), 1.77 (s, 2, purine H-2′ and H-8′), −0.76 (s, 1, C=NOH). The multiplet at τ 4.3 and the singlet at τ −0.76 disappeared, and the multiplet at τ 5.1–4.8 sharpened to a doublet at τ 4.98, $J_{2,3}$ = 9.0 Hz, on addn of D_2O. *Anal.* ($C_{12}H_{16}N_6O_2$) C, H, N.

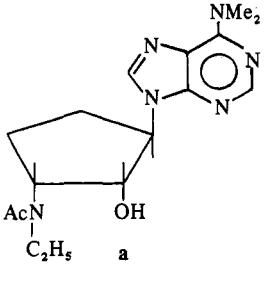
***trans*-2-Acetoxy-3-(6-dimethylamino-9-purinyl)cyclopentanone *O*-Acetyloxime (12).** A soln of 11 (8.95 g, 32.4 mmoles) in Ac_2O (200 ml) was stirred at 60–65° for 4.25 hr. Evapn left a tan solid which was cryst from EtOAc, giving **12** as white crystals (8.16 g, 70%), mp 152–154°; R_f 0.45 in solvent A; ir differs slightly in the fingerprint region from that of the analytical sample. Recrystn of such a sample 3 times from EtOAc gave **12** as white prisms, mp 162.5–164°; R_f 0.45 in solvent A; ir as expected; nmr (DMSO-d_6) τ 8.01 (s, 3, $COCOCH_3$), 7.82 (s, 3, $NOCOCH_3$), 7.7–6.8 (m, overlaps DMSO-d_5, $2CH_2$), 6.53 (s, 6, $N(CH_3)_2$), 5.2–4.4 (m, 1, H-3), 3.50 (d, 1, $J_{2,3}$ = 9.5 Hz, H-2), 1.74 and 1.66 (both s, 2, purine H-2′ and H-8′). *Anal.* ($C_{16}H_{20}N_6O_4$) C, H, N.

Diborane Reduction of 12; Separation of (±)-9-[β-(3α-Acetamido-2α-hydroxy)cyclopentyl]-6-dimethylaminopurine (13) and (±)-9-[α-(3α-Acetamido-2β-hydroxy)cyclopentyl]-6-dimethylaminopurine (14). To a stirred, cooled (0–5°) soln of **12** (4.37 g, 12.1 mmoles) in dry THF (100 ml) was added a 1 *M* soln of BH_3 in THF (44.0 ml, approx 130 mequiv of hydride) over a period of 1.0 hr under N_2. The soln was stirred for an addnl 3.0 hr at 0–5°, and then for 12.0 hr at ambient temp. After cooling the reaction soln (ice bath), H_2O (8.3 ml) was added. The THF was evapd (25°, 0.5 mm), and the residue was stirred with 2 *N* HCl (110 ml) at ambient temp for 3.5 hr. After evapn, the residue was dissolved in MeOH (50 ml) and passed slowly through a column of 100 ml of Amberlite IRA-400 resin (OH^-) packed in MeOH. The basic eluent (500 ml) was evapd, leaving a yellow, gummy solid (3.26 g), which tlc in solvent E indicated to be largely a mixt of amino alcohols (major spot at R_f 0.30, smaller spot at R_f 0.33) and numerous minor contaminants having greater R_f values; ir (KBr) 3250 broad (OH, NH_2), 1600, 1555 cm^{-1} (purine). The amino alcohols could not be separated by prep tlc and appeared to carbonate on standing. This gummy solid was dissolved in Ac_2O and stirred at 60° for 1.75 hr. Evapn left a brown glass (3.74 g) which was chromatogd on a column of silica gel (200 g) packed in $CHCl_3$. Elution with 2% MeOH–$CHCl_3$ (2 l.) gave a pale yellow glass (290 mg), R_f 0.46 in solvent B, which crystd from EtOAc–hexane to a white solid (64 mg), mp 181–183°.‡‡ Elution with 3% MeOH–$CHCl_3$ (3 l.) gave **13** as a pale yellow glass (2.09 g), R_f 0.35 in solvent B, which crystd from EtOAc (1.65 g, 45% from 12), mp 151–152°; ir identical with that of the analytical sample. Recrystn of such a sample from EtOAc gave the analytical sample of 13 as white crystals, mp 151.5–152.5°; uv as expected; ir (KBr) 3300–3050 (OH, NH), 1660, 1640 (amide C=O), 1610, 1555, 1535 (purine, amide); ir ($CHCl_3$) 1660 (amide C=O), 1600 (purine), 1562 cm^{-1} (amide); nmr (DMSO-d_6) τ 8.5–7.6 (m) overlapping 8.08 (s) and DMSO-d_5 (7.3, $2CH_2$ and CH_3CO), 6.49 (s, 6, $N(CH_3)_2$), 6.0–4.9 (m, 3, H-1′, H-2′, and H-3′), 4.63 (d, 1, J = 4.8 Hz, OH), 2.5–2.1 (m, 1, NHC=O), 1.73 and 1.75 (both s, 2, purine H-2 and H-8). The doublet at τ 4.63 and the multiplet at τ 2.5–2.1 disappeared within 5 min of addn of D_2O. *Anal.* ($C_{14}H_{20}N_6O_2$) C, H, N.

Contd elution with 3% MeOH–$CHCl_3$ (1.5 l.) gave fractions contg both 13 and 14 (124 mg, 3%), followed by fractions contg only 14 as an amorphous white solid (247 mg), R_f 0.28 in solvent B. Crystn of this solid from EtOAc gave 14 as white crystals (159 mg, 4%), mp 171–171.5°; uv as expected; ir (KBr) 3250, 3200, 3100 (OH, NH), 1638 (amide C=O), 1600, 1550 (purine, amide); ir ($CHCl_3$): 1660, 1600, 1562 cm^{-1}; nmr (DMSO-d_6) τ 8.5–7.6 (m) overlapping 8.08 (s) and DMSO-d_5 (7.5, $2CH_2$ and CH_3C=O), 6.49 (s, 6, $N(CH_3)_2$), 6.4–5.0 (m, 3, H-1′, H-2′, and H-3′), 4.57 (d, 1, J = 4.0 Hz, OH), 1.82 and 1.72 (both s) overlapped by 1.8–1.7 (m, 3, purine H-2 and H-8 and NHC=O). The doublet at τ 4.57 disappeared immediately on addn of D_2O. Complete exchange of the amide NH required several hours, in contrast to the rapid exchange noted for **13**. *Anal.* ($C_{14}H_{20}N_6O_2$) C, H, N.

The diacetyl derivatives 13a and 14a were obtd when the mixt from the BH_3 redn of 1.00 g (2.78 mmoles) of **12** was stirred in Ac_2O (5 ml)–pyridine (10 ml) at ambient temp for 18 hr. Evapn left

‡‡Spectra of this solid (uv, ir, nmr), a mass spectrum molecular ion of 332, and elemental analysis (C, H, N) suggest a molecular formula of $C_{16}H_{24}N_6O_3$ and the structure a. An O → N acyl migration during the BH_3 redn, followed by redn of the *N*-acyl moiety and subsequent acetylation of treatment with Ac_2O could account for this minor product.



a

a brown glass (800 mg) which was chromatogd on a column of silica gel (50 g) packed in $CHCl_3$. Elution with 1% MeOH–$CHCl_3$ (700 ml) gave 14a as a colorless glass (154 mg), R_f 0.52 in solvent B. Crystn from EtOAc–hexane gave white solid (32 mg, 3%), mp 207.5–210° dec; ir as expected. *Anal.* ($C_{16}H_{22}N_6O_3$) C, H, N.

Elution with 2% MeOH–$CHCl_3$ (850 ml) gave a yellow glass which crystd from EtOAc giving **13a** as tan crystals (377 mg, 39%), mp 194.5–196°, R_f 0.41 in solvent B. Recrystn of such a sample from EtOAc gave the analytical sample of 13a as white needles, mp 194.5–196.5°; ir and nmr as expected. *Anal.* ($C_{16}H_{22}N_6O_3$) C, H, N.

In the chromatog of 13 and 14, as well as 13a and 14a, dark materials remained on the silica gel columns after all of the acetylated compds had been eluted.

The diacetyl derivative 13a was converted to 13 in 85% yield on treatment with a satd soln of NH_3 in MeOH, confirming the assignment of structure of 13a and 14a.

Acyl Migration Studies. Condns were chosen which have been shown to give 63% migration with *cis*-2-benzamidocyclopentanol and no migration with *trans*-2-benzamidocyclopentanol.[27]

A. With 13. The *N*-Ac derivative 13 (50 mg, 0.164 mmole) was dissolved in 0.444 *N* ethanolic HCl (7.43 ml, 3.30 mequiv) and stirred at 25° in a stoppered flask for 18 hr. Evapn left a white solid, mp 100–110° with frothing; ir (KBr) 1737 cm^{-1} (acetate C=O indicates presence of **15**).

B. With 14. Treatment of 14 with ethanolic HCl as in part A gave, on evapn of EtOH, a white solid, mp 95–100° with frothing; ir (KBr) shows no acetate C=O band.

(±)-9-[β-(3α-Amino-2α-hydroxy)cyclopentyl]-6-dimethylaminopurine Acetate (16). The *N*-Ac deriv 13 (730 mg, 2.40 mmoles) was refluxed in a satd aq soln of $Ba(OH)_2$ (approx 0.5 *N*, 40 ml) for 3.0 hr. The soln was dild with EtOH (50 ml) and treated with excess Dry Ice. The $BaCO_3$ was removed by filtration through Celite. Evapn left a white solid (740 mg), mp 180–182° dec, which was resolidified from EtOH–Et_2O, giving **16** as a white powder (685 mg, 89%), mp 184–186° dec; uv and ir as expected; nmr (DMSO-d_6) τ 8.18 (s) overlapped by 8.8–7.7 (m, 7, $CH_3CO_2^-$ and $2CH_2$), 6.51 (s, 6, $N(CH_3)_2$), 5.8–4.2 (m, 3, H-1′, H-2′, and H-3′), 4.5–4.2 (m, 4, NH_3^+ and OH), 1.77 (s, 2, purine H-2 and H-8). The multiplet at τ 4.5–4.2 disappeared on addn of D_2O. *Anal.* ($C_{12}H_{18}N_6O \cdot CH_3CO_2H$) C, H, N.

(±)-9-[β-(3α-Amino-2α-hydroxy)cyclopentyl]-6-dimethylaminopurine 2′,3′-Carbamate (18). The procedure is that used by Baker and Joseph[28] to prepare the 2′,3′-carbamate of the puromycin aminonucleoside. A soln of **16** (100 mg, 0.310 mmole) and Et_3N (0.13 ml, 0.93 mmole) in DMF (5 ml) was cooled to 5°. Carbobenzoxy chloride (0.05 ml, approx 0.5 mmole) was added, and the soln was stirred at ambient temp for 1.0 hr. H_2O (20 ml) was added, and the oil which formed was extd into $CHCl_3$ (3 × 20 ml). The $CHCl_3$ exts were dried ($CaSO_4$) and evapd, leaving a colorless glass (96 mg), which tlc in solvent B showed to be a mixt of 17 (R_f 0.50) and 18 (R_f 0.28) in approx equal amts. When the mixt of 17 and 18 was treated with NaOMe in DMF as described below, 18 was isolated in 74% yield (from 16). In another run 17 and 18 were separated on a silica gel column eluted with 3% MeOH–$CHCl_3$. The Cbz derivative 17 was eluted as a glass which crystd from abs EtOH, giving white crystals, mp 163–164.5°; ir and nmr as expected. *Anal.* ($C_{20}H_{24}N_6O_3$) C, H, N.

Contd elution of the column gave 18 as a white solid, mp 223–224°; ir identical with that of the analytical sample prepd as described below.

A sample of 17 purified by chromatog (890 mg, 2.25 mmoles) was dissolved in DMF (10 ml), and 0.30 ml (0.4 mmole) of an approx 1.4 *N* soln of NaOMe in MeOH was added. The soln was stirred at 100° for 2.0 hr. At this time tlc showed complete conversion to **18**. Evapn left 18 as a glass which solidified from abs EtOH (10 ml), giving a white powder (452 mg, 70%), mp 218–220°. Crystn from abs EtOH gave white crystals, mp 226–227°; ir (KBr) 3275 broad (NH), 1757 and 1733 (carbamate C=O), ir ($CHCl_3$) single carbamate band at 1760; nmr as expected. *Anal.* ($C_{13}H_{16}N_6O_2$) C, H, N.

9-{*R*-[3*R*-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-2*R*-hydroxy]cyclopentyl}-6-dimethylaminopurine (2a) and 9-{*S*-[3*S*-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-2*S*-hydroxy]cyclopentyl}-6-dimethylaminopurine (19a). **Method A.**[21,22] A soln of 16 (145 mg, 0.450 mmole) in MeOH was passed slowly through a column of 10 ml of Amberlite IRA-400 resin (OH^-) packed in MeOH. Evapn left the free amine as a white solid (118 mg, 0.450 mmole), mp 153–154°, which was dissolved immediately in DMF (5 ml), along with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[4] (155 mg, 0.472 mmole) and *N*-hydroxysuccinimide (54.3 mg, 0.472 mmole). The soln was cooled to 0°, and DCC (97.4 mg, 0.472 mmole) was added. After stirring at 0° for 30 min, the soln was allowed to stir at ambient temp for 20 hr. The mixt was filtered, the dicyclohexylurea was washed with EtOAc (20 ml), and the combined filtrate was evapd. A soln of the residue in EtOAc (5 ml) was cooled to 0° and then filtered. The filtrate was dild to 15 ml with EtOAc, extd with H_2O (2.5 ml), then one-half satd $NaHCO_3$ (2.5 ml), then H_2O (2 × 5 ml). Evapn of the dried EtOAc soln left a mixt of 2a and 19a as a white solid foam (250 mg, 0.436 mmole, 97%), R_f 0.52 in solvent B. The diastereomers could not be sepd by crystn or chromatog. An analytical sample of the mixt was prepd by solidification from MeOH, giving white powder; mp: part at 170–172° and the rest at 200–205°; ir and nmr as expected. The purine and methoxy nmr resonances indicate a mixt. *Anal.* ($C_{30}H_{35}N_7O_5$) C, H, N.

Method B.[23] A soln of Et_3N (61.3 mg, 0.606 mmole) in dry THF (10 ml) was cooled to −10°. Ethyl chlorocarbonate (72.4 mg, 0.667 mmole) was added. The soln was stirred for 1 min, and then *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[4] (200 mg, 0.606 mmole) was added and the mixt stirred at −10° for 10 min. A sample of the free amine obtd by resin treatment of 16 as in method A (159 mg, 0.606 mmole) was dissolved in DMF, and the soln was cooled to −10° and added to the mixed anhydride. The resulting mixt was stirred at −10° for 1.0 hr and then stored at 4° for an addnl 24 hr. It was filtered, and the solid collected was washed with addnl DMF (5 ml). Evapn of the combined filtrates left a colorless glass which was triturated with H_2O (5 ml), evapd to dryness, and chromatogd by prep tlc on a plate developed in solvent C. Extn of the major band with 20% MeOH–$CHCl_3$ (other bands were unreacted starting materials) gave a mixt of 2a and 19a as a solid foam (266 mg, 77%), R_f, ir, and nmr identical with those of the mixt obtained from method A.

The crude mixt of amino alcohols resulting from the diborane redn of 3.00 g (8.32 mmoles) of 12 was treated directly with the mixed anhydride of *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine in this manner, giving a mixt of 2a and 19a, after chromatog, in 30% yield (from 12); R_f, ir, nmr identical with those of the samples described above.

6-Dimethylamino-9-{*R*-[2*R*-hydroxy-3*R*-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl}purine (2) and 6-Dimethylamino-9-{*S*-[2*S*-hydroxy-3*S*-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl}-purine (19). A mixt of 2a and 19a prepd by method A (250 mg, 0.436 mmole) was dissolved in glacial AcOH (15 ml) and shaken with 10% Pd/C (125 mg) under H_2 (1 atm) for 10 min, by which time H_2 uptake had ceased. The mixt was filtered through Celite and the Celite was washed with addnl AcOH (10 ml). Evapn of the combined filtrate and wash (35°, 0.5 mm) left a colorless glass. A soln of this glass in MeOH was passed slowly through a column of 10 ml of Amberlite IRA-400 resin (OH^-) packed in MeOH. The basic MeOH eluent (250 ml) was evapd, leaving a mixt of 2 and 19 as a white solid (176 mg, 92%), mp 194–196°; tlc gave 2 spots with R_f 0.41 and 0.51 in solvent C; tlc and ir identical with those of the analytical sample. Recrystn from MeOH (5 ml) gave a 1:1 mixt of 2 and 19 as white crystals, mp 195–196°; $[\alpha]_{589}$ −45.6°, $[\alpha]_{436}$ −103.8° (*c* 0.26, $CHCl_3$); uv max (0.1 *N* HCl) 270 mμ (log ε 4.307); (H_2O) 277 (4.314); (0.1 *N* NaOH) 277 (4.312); ir (KBr) 3420, 3310 (OH, NH_2, NH), 1670 and 1655 (2 amide C=O), 1600 cm^{-1} (arom, NH_2); ir ($CHCl_3$) 1660 (2 amide, C=O), 1605 (arom, NH_2); nmr identical with that of pure 19. *Anal.* ($C_{22}H_{29}N_7O_3$) C, H, N. Further recrystn of such a mixt did not change the ratio of **2** to **19**.

The diastereomers 2 and 19 were sepd by prep tlc (50–70 mg per plate) in solvent D. The 2 bands were each stirred for 18 hr with 20% MeOH–$CHCl_3$, filtered, and evapd, giving almost quant recovery of the pure diastereomers as colorless glasses. The glass having R_f 0.51, assigned structure **19**, crystd from abs EtOH, giving white needles, mp 161–161.5°; $[\alpha]_{589}$ −8.1°, $[\alpha]_{436}$ −16.2° (*c* 0.43, $CHCl_3$); uv max (0.1 *N* HCl) 270 mμ (log ε 4.301); (H_2O) 277 (4.323); (0.1 *N* NaOH) 277 (4.322); ir (KBr) 3417, 3309, 3300–3100 (OH, NH_2, NH), 1655 (amide C=O), 1600 cm^{-1} (aromatic, NH_2); ir ($CHCl_3$) 1650, 1600; nmr (DMSO-d_6) τ 8.7–8.2 (m, 2, NH_2), 8.2–7.0 (m, 6, 3 CH_2), 6.52 (s, 7, $N(CH_3)_2$ and NCHC=O), 6.25 (s, 3, OCH_3), 6.0–5.0 (m, 3, H-1′, H-2′, and H-3′), 4.7–4.2 (m, 1, OH), 2.95 (q, 4, OC_6H_4), 2.3–1.9 (m, 1, NHC=O), 1.73 (s, 2, purine H-2 and H-8). The multiplets at τ 8.7–8.2, 4.7–4.2, and 2.3–1.9 disappeared on addn of D_2O; mass spectrum (probe temp *ca.* 250°) *m/e* above 80 (relative intensity) 439 (0.6), 422 (0.8), 421 (1.2), 420 (0.6), 419 (0.5), 418 (0.5), 405 (0.7), 404 (1.8), 403 (0.9), 318 (23.6), 301 (21.4), 300 (100), 271 (8.6), 228 (11.9), 190 (12.5), 165 (8.1), 164 (82.4), 163 (16.5), 150 (14.8), 148 (9.4), 134 (23.3), 122 (10.3), 121 (59.6), 120 (5.6), 109 (5.7), 82 (9.8); metastable transitions: 283.0 (283.0 calcd for $318^+ \rightarrow 300^+$), 173.5 (173.3 calcd for $300^+ \rightarrow 228^+$), 110 (110.2 calcd for $163^+ \rightarrow 134^+$), 89.7 (89.7 calcd for $300^+ \rightarrow 164^+$). *Anal.* ($C_{22}H_{29}N_7O_3$) C, H, N.

The glass having R_f 0.41, assigned structure **2**, could not be

crystd. Drying at 56° (0.05 mm) for 24 hr gave a white solid foam; $[\alpha]_{589}$ −82.6°, $[\alpha]_{436}$ −188.4° (*c* 0.14, $CHCl_3$); uv max (0.1 *N* HCl) 270 mμ (log ε 4.308); (H_2O) 277 (4.317); (0.1 *N* NaOH) 277 (4.317); ir (KBr) 3375 broad (OH, NH_2, NH), 1650 (amide C=O), 1590 cm^{-1} (arom, NH_2); ir ($CHCl_3$) 1650, 1597; mass spectrum (probe temp *ca.* 260°), *m/e* above 80 (relative intensity) 439 (1.0), 422 (1.6), 421 (1.5), 420 (1.8), 419 (3.7), 418 (2.7), 405 (1.0), 404 (1.8), 403 (1.1), 319 (6.5), 318 (34.2), 301 (22.6), 300 (100), 289 (6.5), 271 (8.8), 228 (11.8), 190 (17.5), 165 (7.7), 164 (76.8), 163 (17.5), 150 (13.2), 148 (12.3), 134 (23.2), 122 (6.7), 121 (39.0), 120 (5.4), 109 (4.1), 82 (9.8), metastable transitions same as those of **19**. *Anal.* ($C_{22}H_{29}N_7O_3$) C, H, N.

Hydrogenolysis of a mixt of **2a** and 19a prepd by method B gave a 91% yield of 2 and 19 which, after sepn by chromatog, had [α] within experimental error of those of samples prepd by method A.

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Identification and Synthesis of the Major Nucleoside Metabolite in Rabbit Urine after Administration of Puromycin Aminonucleoside[1]†

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9-(3′-Amino-3′-deoxy-β-D-ribofuranosyl)-6-dimethylamino-9*H*-purine (1a), the aminonucleoside of puromycin, when administered to rabbits is monodemethylated at the 6-N position to give 9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-6-methylamino-9*H*-purine (**9**), the latter constituting the major nucleoside metabolite of 1a in the urine. The 3′-N-acetylated derivative of the metabolite, **9**, *i.e.*, 9-(3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-6-methylamino-9*H*-purine (**8**) was identical in all respects (tlc patterns, degradation products, mass spectral fragments) to **8** synthesized chemically by methylation of 9-(3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-6-amino-9*H*-purine (**6**) on the 1 position with MeI, followed by rearrangement in dil NH_4OH. Contrary to earlier speculations, rabbits do not metabolize 1a by methylation on the 3′-amino group of the amino ribose moiety, as shown by comparison of the urinary metabolites of 1a with chemically synthesized 3′-N-methylated derivatives of 1a, *viz.*, 9-(3′-methylamino-3′-deoxy-β-D-ribofuranosyl)-6-dimethylamino-9*H*-purine (1c), and 9-(3′-dimethylamino-3′-deoxy-β-D-ribofuranosyl)-6-dimethylamino-9*H*-purine (1b).

The aminonucleoside **1a** produced by the Edman degradation of the antibiotic puromycin and independently synthesized, by Baker, *et al.*,[2,3] exhibits trypanocidal as well as antitumor properties.[4,5] The appearance of massive, though reversible, proteinuria frustrated clinical trials of **1a** as a tumor chemotherapeutic agent in man.[6] When administered to rats by oral, sc, or ip routes, **1a** elicits a nephrotic syndrome characterized by hypoproteinemia, hyperlipidemia, hypercholesterolemia, proteinuria, edema, and ascites—a syndrome that is clinically indistinguishable from the kidney disease frequently observed in children.[7] **1a** has since been utilized extensively for the experimental induction of this disease in rats.[8]

The striking species susceptibility to toxicity by **1a** manifested by its lack of nephrotoxicity in mice, guinea pigs, or rabbits, does not appear to be reflected in differential

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