Synthesis and Biological Evaluation of 5'-Sulfamoylated Purinyl Carbocyclic Nucleosides

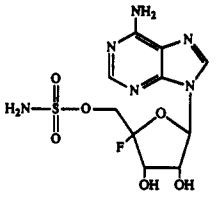
Eileen M. Peterson, Jay Brownell, and Robert Vince*

Department of Medicinal Chemistry, College of Pharmacy, 308 Harvard Street S.E., University of Minnesota, Minneapolis, Minnesota 55455-0343

Received July 2, 1992

The first series of 5'-sulfamoylated carbocyclic purinyl nucleosides was synthesized and tested for antitumor and antibacterial activities. The target compounds were formed by reacting the 2',3'-acetonide-protected carbocyclic nucleosides with sulfamoyl chloride, followed by deprotection. The agents were tested for cytotoxic activity against P388 mouse leukemia cells. Two compounds, 5'-sulfamoyl carbocyclic adenosine (2) and 5-sulfamoyl-8-aza carbocyclic adenosine (6) exhibited IC_{50} values as low as 62 and 15 nM, respectively. These analogs inhibited protein biosynthesis and slowed down DNA and RNA biosyntheses in the P388 cells. None of the target molecules were as potent against *Eschericia coli* as they were against the tumor cells. Also, in cell-free systems, agents 2 and 6 were more effective inhibitors of protein synthesis in rabbit reticulocyte lysate than in *E. coli*. These new carbocyclic derivatives appear to be somewhat selective for eukaryotic over prokaryotic cells in affecting translation.

Nucleocidin (1), a nucleoside antibiotic produced by the bacteria *Streptomyces calvus*,[1,2] is active against a wide variety of Gram-positive bacteria, gram negative bacteria and trypanosomes.[3] However, its high toxicity has prevented its therapeutic use. The structure of nucleocidin differs from the structure of other natural nucleosides in that it contains 4'-fluoro and 5'-sulfamoyl groups.[4] Since the discovery of nucleocidin, many defluorinated analogs containing the 5'-sulfamoyl group have been examined. These agents have been shown to exhibit antibacterial,[5,8,9] antitumor,[5,6] antiviral,[10,12-14] antiparisitic,[7,11,15] and herbicidal[8] activities.



Nucleocidin (1)

In this study, the first series of carbocyclic 5'-sulfamoylated nucleosides is reported, and the new agents are evaluated for antibacterial and antitumor activities. Carbocyclic nucleosides, nucleosides in which the sugar ring oxygen has been replaced with a methylene group, are very similar in structure to the natural derivatives; thus, many carbocyclic agents are recognized by nucleoside and nucleotide kinases and are subsequently phosphorylated.[16] The mono-, di-, and triphosphorylated products formed in these reactions are often effective antitumor and antiviral agents.[16f] Carbocyclic adenosine (C-Ado), for example, forms a monophosphate capable of inhibiting at least one enzyme in purine de novo biosynthesis.[17] This

(1) Backus, E. J.; Tresner, H. D.; Campbell, T. H. The Nucleocidin and Alazopeptin Producing Organisms: Two New Species of *Streptomyces*. *Antibiot. Chemother.* **1957**, *7*, 532-541.

(2) Thomas, S. O.; Singleton, V. L.; Lowery, J. A.; Sharpe, R. W.; Pruess, L. M.; Porter, J. N.; Mowat, J. H.; Bohonos, N. Nucleocidin, a New Antibiotic with Activity against Trypanosomes. *Antibiot. Annu. 1956-57* **1957**, 716-721.

(3) (a) Florini, J. R. Nucleocidin. In *Antibiotics*; Gottlieb, D., Shaw, P. D., Eds.; Springer-Verlag: New York, 1967; pp 427-433. (b) Suhadolnik, R. J. *Nucleoside Antibiotics*; John Wiley & Sons, Inc.: New York, 1970; pp 246-256.

(4) Morton, G. O.; Lancaster, J. E.; Van Lear, G. E.; Fulmor, W.; Meyer, W. E. *The Structure of Nucleocidin*. III (a New Structure). *J. Am. Chem. Soc.* **1969**, *91*, 1535-1537.

(5) Block, A.; Coutsogeorgopoulos, C. Inhibition of Protein Synthesis by 5'-Sulfamoyladenosine. *Biochemistry* **1971**, *10*, 4394-4398.

(6) Klein, E.; Burgess, G. H.; Bloch, A.; Milgrom, H.; Holtermann, O. A. The Effects of Nucleoside Analogs on Cutaneous Neoplasms. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 216-224.

(7) Jaffe, J. J.; McCormack, J. J.; Meymerian, E. Trypanocidal Properties of 5'-O-Sulfamoyladenosine, a Close Structural Analog of Nucleocidin. *Exp. Parasitol.* **1970**, *28*, 535-543.

(8) Iwata, M.; Sasaki, T.; Iwamatsu, H.; Miyadoh, S.; Tachibana, K.; Matsumoto, K.; Shomura, T.; Sezaki, M.; Watanabe, T. A New Herbicidal Antibiotic, SF2494 (5'-O-sulfamoyltubercidin) Produced by *Streptomyces mirabilis*. *Meiji Seika Kenkyu Nenpo.* **1987**, *26*, 17-22.

(9) Takahashi, E.; Beppu, T. A. New Nucleosidic Antibiotic AT265. *J. Antibiot.* **1082**, *35*, 939-947.

(10) (a) Smee, D. F.; Alaghamandan, H. A.; Kini, G. D.; Robins, R. K. Antiviral activity and mode of action of ribaviron 5'-sulfamate against Semliki Forest virus. *Antiviral Res.* **1988**, *10*, 253-262. (b) Smee, D. Antiviral Activity of the Nucleotide Analogue Ribavirin 5'-Sulfamate. In *Nucleotide Analogs as Antiviral Agents, ACS Symposium Series 401*; Martin, J., Ed.; American Chemical Society: Washington, D.C., 1989; pp 124-139.

(11) Kini, G. D.; Henry, E. M.; Robins, R. K.; Larson, S. B.; Marr, J. J.; Berens, R. L.; Bacchi, C. J.; Nathan, H. C.; Keithly, J. S. Synthesis, Structure, and Antiparasitic Activity of Sulfamoyl Derivatives of Ribavirin. *J. Med. Chem.* **1990**, *33*, 44-48.

(12) Camarasa, M. J.; Fernandez-Resa, P.; Garcia-Lopez, M. T.; de Las Heras, F. G.; Mendez-Castrillon, P. P.; Alarcon, B.; Carrasco, L. Uridine 5'-Diphosphate Glucose Analogues. Inhibitors of Protein Glycosylation That Show Antiviral Activity. *J. Med. Chem.* **1985**, *28*, 40-46.

(13) Castro-Pichel, J.; Garcia-Lopez, M. T.; de Las Heras, F. G.; Herranz, R. Synthesis and Antiviral Activity of 5'-O'-(Substituted) Sulfamoyl Pyrimidine Nucleosides. *Arch. Pharm. (Weinheim, Ger.)* **1989**, *322*, 11-15.

(14) Vilas, P.; Perez, C.; Perez, S.; Villalon, D. G.; Gancedo, A. G.; Gil-Fernandez, C.; Garcia-Lopez, M. T.; de las Heras, F. G. Effect of a Uridine 5'-Diphosphate Glucose Analogue on Herpes simplex keratitis in Rabbits and Vaginal Infection in Guinea Pigs. *Chemotherapy* **1989**, *35*, 58-63.

(15) Alcina, A.; Fresno, M.; Alarcon, B. Activity of P536, a UDP-Glucose Analog, against *Trypanosoma cruzi*. *Antimicrob. Agents Chemother.* **1988**, *32*, 1412-1418.

(16) (a) Suhadolnik, R. J. *Nucleoside Antibiotics*; John Wiley & Sons, Inc.: New York, 1970. (b) Goodchild, J. The Biochemistry of Nucleoside Antibiotics. *Top Antibiot. Chem.* **1982**, *6*, 99-228. (c) Buchanan, J. G.; Wightman, R. H. The Chemistry of Nucleoside Antibiotics. *Top. Antibiot. Chem.* **1982**, *6*, 229-323. (d) Montgomery, J. A. Studies on the Biologic Activity of Purine and Pyrimidine Analogs. *Med. Res. Rev.* **1982**, *2*, 271-308. (e) Marquez, V. E.; Lim, M.-I. Carbocyclic Nucleosides. *Med. Res. Rev.* **1986**, *6*, 1-40. (f) Montgomery, J. A. Approaches to Antiviral Chemotherapy. *Antiviral Res.* **1989**, *12*, 113-132.

0022-2623/92/1835-3991$03.00/0

inhibition may contribute to C-Ado's in vitro antitumor activity. Carbocyclic nucleosides also have metabolic features which make them attractive for in vivo use. These compounds are resistant to the actions of purine nucleoside phosphorylases (PNP's),[18] and they are poorer substrates than natural nucleosides for adenosine deaminases (ADA's).[16e,19] Because of this resistance to normal purine metabolism, carbocyclic nucleosides may have longer in vivo half-lives than natural sugar nucleosides and therefore could yield drugs with longer durations of action.

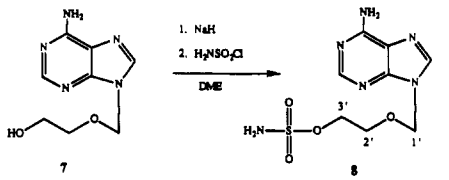
The carbocyclic agents chosen for this investigation all contain an adenine or adenine-like base. This choice was based on the results of a study by Bloch and Coutsogeorgopoulos[5] where defluorinated analogs of nucleocidin containing adenine, inosine, cytidine, uracil, and thymine as the bases attached to a 5′-sulfamoylated sugar ring were studied. This study revealed that the adenine base was necessary for antibacterial and antitumor activities. Thus, the target compounds all consist of an adenine (2) or adenine-like (3–6) base combined with a 5′-sulfamoylated carbocyclic sugar ring (Table I). The 6-position of purine ring in these compounds was explored by replacing the amino group with *N*-methyl (3), *N,N*-dimethyl (4), and *S*-methyl (5) groups. An 8-azaadenine base (6) was also attached to the carbocyclic ring since 8-azapurines have often exhibited greater antitumor activity than the natural purine agents.[16d] Of the new agents studied, two (2 and 6) displayed extremely potent antitumor activity, and therefore preliminary studies on their mechanisms of action were performed.

Chemistry

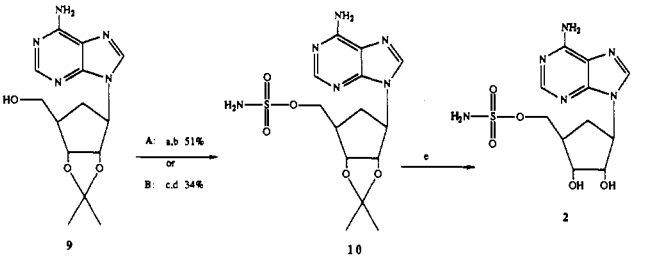
The general synthetic procedure involved the reaction of sulfamoyl chloride with 2′,3′-acetonide-protected carbocyclic nucleosides followed by deprotection. In the synthesis of 5′-sulfamoyladenosine by Shuman et al.,[20] the sulfamoylation was effected by activation of the 5′-hydroxyl on the orthoester-protected nucleoside with sodium hydride followed by reaction with sulfamoyl chloride. In the synthesis of nucleocidin, Jenkins et al. activated the acetonide-protected nucleoside by reacting with bis(tributyltin) oxide and followed with the addition of sulfamoyl chloride.[21]

As a model reaction, acycloadenosine (7) originally reported by Schaeffer et al.,[22] was reacted with sodium hydride and sulfamoyl chloride, and this produced 9-[[2-(sulfamoyloxy)ethoxy]methyl]adenosine (8) (Scheme I). Likewise, when 2′,3′-isopropylidene carbocyclic adenosine

Scheme I

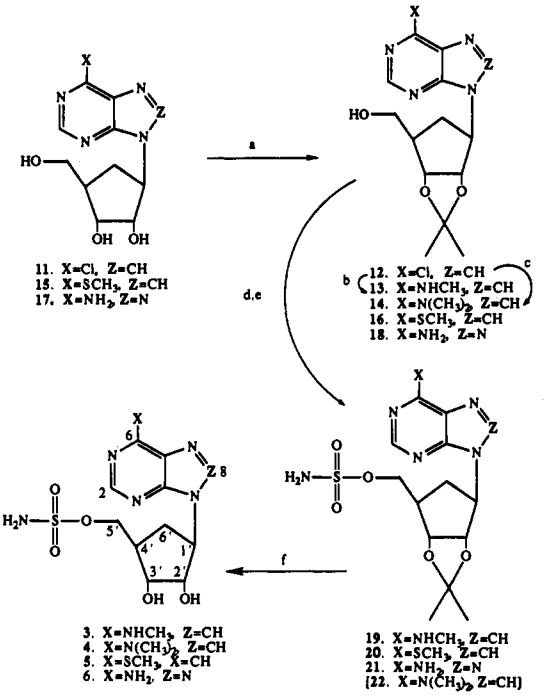


Scheme II [a]



[a] (a) NaH, DME; (b) H_2NSO_2Cl, DME; (c) $[(n\text{-}Bu)_3Sn]_2O$, benzene; (d) H_2NSO_2Cl, dioxane; (e) CF_3CO_2H, H_2O.

Scheme III [a]



[a] (a) acetone, *p*-TsOH, 2,2-dimethoxypropane; (b) 40% NH_2CH_3 in H_2O, 85 °C; (c) 40% $NH(CH_3)_2$, 100 °C; (d) NaH, DME; (e) H_2NSO_2Cl, DME; (f) CF_3CO_2H, H_2O.

(9), first reported by Shealy et al.,[23] was reacted under these conditions, intermediate 10 was isolated in a 52% yield (Scheme II). When 9 was activated with bis(tributyltin) oxide and then reacted with sulfamoyl chloride, 10 was obtained in a 34% yield. Because of the better yield obtained with sodium hydride, this method was used for all further sulfamoylations. Compound 10 was easily deprotected in aqueous trifluoroacetic acid to give the first target molecule 2.

The 6-chloropurinyl carbocyclic nucleoside[24] 11, was protected as the acetonide to give 12 (Scheme III). The 6-chloro group in 12 was easily displaced with methylamine

(17) Hill, D. L.; Straight, S.; Allan, P. W.; Bennett, Jr., L. L. Inhibition of Guanine Metabolism of Mammalian Tumor Cells by the Carbocyclic Analogue of Adenosine. *Mol. Pharmacol.* 1971, *7*, 375–380.

(18) Doskocil, J.; Holy, A. Specificity of Purine Nucleoside Phosphorylase From *Escherichia coli*. *Collect. Czech. Chem. Commun.* 1977, *42*, 370–383.

(19) Bennett, L. L., Jr.; Allan, P. W.; Hill, D. L. Metabolic Studies with Carbocyclic Analogs of Purine Nucleosides. *Mol. Pharmacol.* 1968, *4*, 208–217.

(20) (a) Shuman, D. A.; Robins, M. J.; Robins, R. K. The Synthesis of Adenine 5′-O-Sulfamoyl Nucleosides Related to Nucleocidin. *J. Am. Chem. Soc.* 1969, *91*, 3391–3392. (b) Shuman, D. A.; Robins, M. J.; Robins, R. K. The Synthesis of Nucleoside Sulfamates Related to Nucleocidin. *J. Am. Chem. Soc.* 1970, *92*, 3434–3440.

(21) (a) Jenkins, I. D.; Verheyden, J. P. H.; Moffatt, J. G. Synthesis of the Nucleoside Antibiotic Nucleocidin. *J. Am. Chem. Soc.* 1971, *93*, 4323–4324. (b) Jenkins, I. D.; Verheyden, J. P. H.; Moffatt, J. G. 4′-Substituted Nucleosides. 2. Synthesis of the Nucleoside Antibiotic Nucleocidin. *J. Am. Chem. Soc.* 1976, *98*, 3346–3357.

(22) Schaeffer, H. J.; Gurwara, S.; Vince, R.; Bittner, S. Novel Substrate for Adenosine Deaminase. *J. Am. Chem. Soc.* 1971, *14*, 367–369.

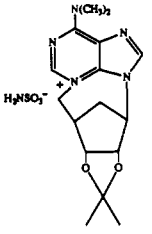
(23) Shealy, Y. F.; Clayton, J. D. Synthesis of Carbocyclic Analogs of Purine Ribonucleosides. *J. Am. Chem. Soc.* 1969, *91*, 3075–3083.

(24) Shealy, Y. F.; Clayton, J. D. Carbocyclic Analogs of 6-Substituted Purine Ribonucleosides and of Adenosine Ribonucleotides. *J. Pharm. Sci.* 1973, *62*, 1252–1257.

and dimethylamine to produce intermediates 13 and 14, respectively. The 6-(thiomethyl) derivative was approached by first forming the 6-thiopurine from 6-chloropurine intermediate 12, followed by methylation. However, in one experiment, displacement of the chlorine with a thiol using thiourea in refluxing propanol effected not only the formation of the 6-thiopurine, but also the deprotection of the acetonide. A potential cause for this deprotection could be trace amounts of acid and water in the propanol used. Because of this unusual result, the 6-(thiomethyl) nucleoside 15 was synthesized from 11 as described by Shealy.[23] The diol in 15 was protected with an acetonide to give 16. 8-Aza carbocyclic adenosine[25] (17), was similarly protected to give acetonide 18.

Treatment of methylamino (13), methylthio (16), and 8-azaadenosine (18) derivatives with sodium hydride and sulfamoyl chloride furnished the sulfamoylated protected intermediates 19–21, respectively. Deprotection of the diols in aqueous trifluoroacetic acid provided the three target molecules 3, 5, and 6, respectively (Scheme III).

The dimethylamino derivative 14 was also reacted with sodium hydride and sulfamoyl chloride; however, a pure sample of the sulfamoylated intermediate 22 was never isolated (Scheme III). The proton NMR spectrum of the impure product indicated that some anhydro nucleoside (23) may have formed via N-3,C-5' cyclization. Normally, H2 and H8 of the purine ring in N^6,N^6-dimethyl bases occur at ~8.28 and ~8.21 ppm in dimethyl sulfoxide (as in compound 14). However, when an anhydro derivative forms, these protons are shifted downfield. For example, in adenosine the shift occurs from 7.9 and 8.25 to 8.66 and 8.78.[26] In the impure sample of 22, peaks were seen at 8.67 and 8.48 ppm in addition to the normal purine peaks at 8.28 and 8.21, suggesting an anhydro impurity. Triethylamine was therefore explored as a 5'-hydroxyl activating agent to see if this weaker base could somehow offset the N-3 to C-5' cyclization. However, the intermediate 22 produced using triethylamine contained many impurities. Deprotection of the impure sample yielded target molecule 4 in a 33% yield from 14. Both sodium hydride and triethylamine conditions led to compound 4; however, the best yield was obtained when triethylamine was used in the reaction sequence.

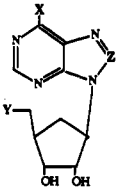


23

Biological Results and Discussion

The compounds in Table I were evaluated for cytotoxicity against P388 mouse leukemia cells.[27] Puromycin, a protein biosynthesis inhibitor and a potent antitumor agent, was used as the positive control in the antitumor assay. Compound 2 and the corresponding 8-aza analog 6 manifested very potent activity (IC_{50}'s of 10^{-1} to 10^{-2} μg/mL). Agent 3 was also quite toxic, possessing an IC_{50} of 2–4 μg/mL, while the acyclo derivative 8 was nontoxic. The other new agents 4 and 5, exhibited some toxicity, but significantly less than agents 2 and 6. Carbocyclic adenosine (C-Ado) and 8-aza carbocyclic adenosine (17), known potent antitumor agents, were tested to compare their activities with agents 2 and 6. The 5'-sulfamoylated compounds 2 and 6 were found to be significantly more potent than 5'-hydroxylated agents C-Ado and 17; thus, the activity of the new sulfamoylated derivatives was not a result of hydrolysis of the 5'-sulfamoyl group and the release of C-Ado or 17.

Table I. Cytotoxicity to P388 Mouse Leukemia Cells



	X	Z	Y	IC_{50} (μg/mL)
2	NH_2	CH	OSO_2NH_2	0.062–0.125
3	$NHCH_3$	CH	OSO_2NH_2	2–4
4	$N(CH_3)_2$	CH	OSO_2NH_2	~74
5	SCH_3	CH	OSO_2NH_2	~50
6	NH_2	N	OSO_2NH_2	0.0156–0.0625
8 (acyclo)	NH_2	CH	OSO_2NH_2	>100
C-Ado	NH_2	CH	OH	~0.5
17	NH_2	N	OH	0.25–0.5
puromycin	-	-	-	0.25–0.5

The in vitro antitumor results revealed that compounds 2 and 6 were very potent cytotoxic agents and, thus, these compounds were worthy of further investigation. Therefore, the mechanism of action of these agents was explored. In assays using the precursors [^{14}C]uridine for RNA, [^{14}C]-thymidine for DNA, and [^{14}C]-L-phenylalanine, [^{14}C]-L-leucine and [^{14}C]-L-lysine for protein, the biosyntheses of these macromolecules were investigated. Figures 1 and 2 depict the results of these studies. Agents 2 and 6 were found to slow down both DNA and RNA biosyntheses; however, after a short lag time, protein biosynthesis was completely shut down by these agents. C-Ado, on the other hand, was found to slow down protein and RNA biosyntheses, but with a more pronounced effect on the RNA biosynthesis (Figure 3). Since agent 2 and C-Ado apparently have different mechanisms of action, this finding further confirms the hypothesis that the toxicity of agent 2 is not due to in vitro hydrolysis to C-Ado.

The whole cell studies using the P388 cells suggested that protein biosynthesis inhibition was a major target for agents 2 and 6. Therefore, a cell free protein biosynthesis system, i.e. an *E. coli* ribosomal system, was explored to determine if (1) these agents were indeed directly inhibiting protein biosynthesis and (2) at what point in this process of translation these agents were interfering. The biosynthesis of polyphenylalanine was looked at, again using puromycin as the positive control. The results of this

(25) (a) Shealy, Y. F.; Clayton, J. D. Carbocyclic Analog of Purine Ribonucleosides with Antileukemic Activity. *J. Pharm. Sci.* 1973, *62*, 858–859. (b) Shealy, Y. F.; Clayton, J. D. Cyclopentyl Derivatives of 8-Azahypoxanthine and 8-Azaadenine. Carbocyclic Analogs of 8-Azainosine and 8-Azaadenosine. *J. Heterocycl. Chem.* 1973, *10*, 601–605.

(26) Townsend, L. B. Nuclear Magnetic Resonance Spectroscopy in the Study of Nucleic Acid Components and Certain Related Derivatives. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. N., Tipson, R. S., Eds.; John Wiley and Sons: New York, 1973; Vol. 2, pp 267–398.

(27) Geran, R. I.; Greenberg, N. H.; McDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Protocols* for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems (Third Edition). *Cancer Chemother. Rep., Part 3* 1972, *3*, 1.

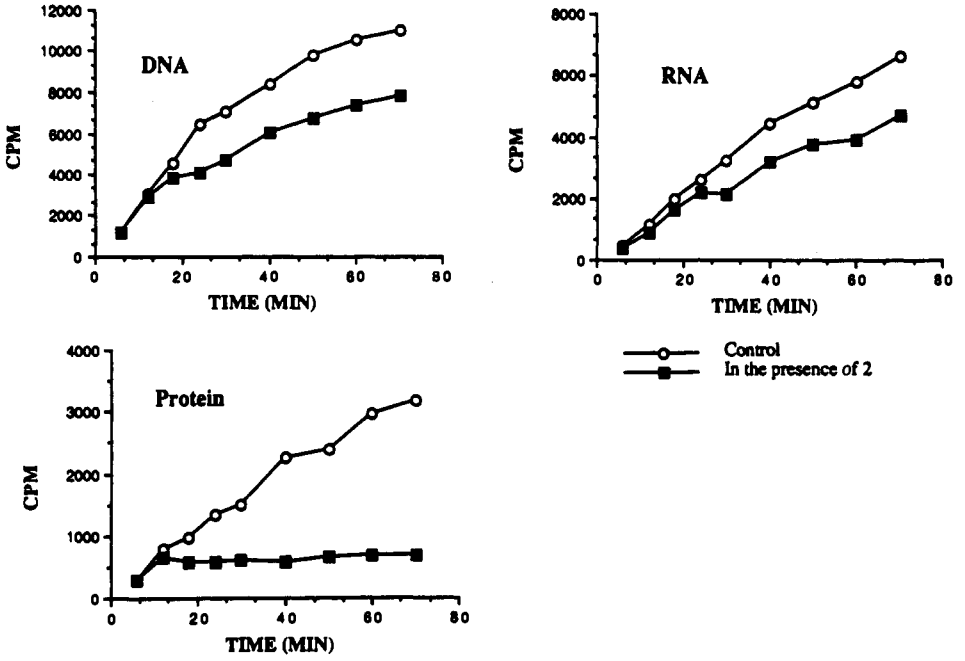


Figure 1. Biosynthesis of DNA, RNA, and protein in P388 mouse leukemia cells in the presence of 30 μM concentration of agent 2.

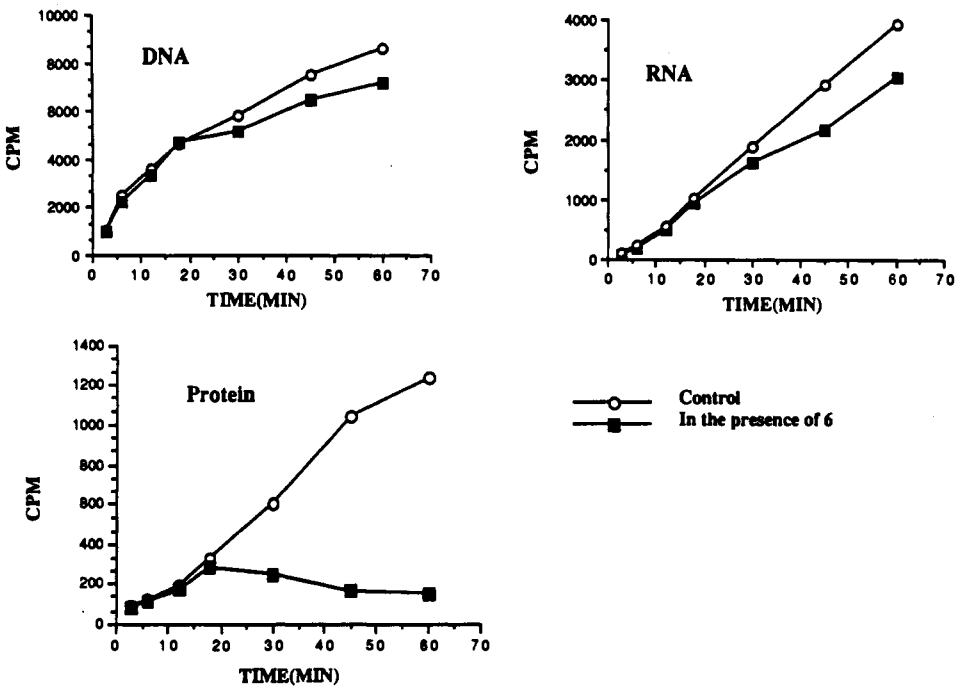


Figure 2. Biosynthesis of DNA, RNA, and protein in P388 mouse leukemia cells in the presence of 12 μM concentration of agent 6.

study, represented in Figure 4, however, indicated that neither compound 2 nor compound 6 inhibited protein biosynthesis to any great extent except at very high concentrations. At concentrations of 1×10^{-4} M, agents 2 and 6 inhibited polyphenylalanine synthesis by approximately 40%. On the other hand, puromycin, an agent known to act directly on protein biosynthesis, inhibited the synthesis by 89% at a concentration of 3×10^{-6} M. Thus, this study suggested that (1) protein biosynthesis may not be directly affected by agents 2 and 6 or, (2) these agents were selective for eukaryotic systems.

Since protein biosynthesis in the *E. coli* ribosomal system appeared to be unaffected by agents 2 and 6, a prokaryotic whole cell system, *E. coli* bacteria, was therefore investigated. The *E. coli* was incubated in the presence of each test agent, and bacterial growth was measured by optical density. Chloramphenicol, a prokaryotic-specific protein biosynthesis inhibitor, ws used as a positive control. The results of this assay are shown in Figure 5. None of the new agents (2–6, 8) exhibited significant antibacterial activity except at very high concentrations. These results support the hypothesis that agents 2 and 6 are selectively toxic to eukaryotic systems.

In order to further confirm that agents 2 and 6 were acting on eukaryotic protein biosynthesis, a rabbit reticulocyte lysate system was explored. By incubating this system with a radioactive amino acid and agent 2 or agent 6, the effectiveness of these agents at inhibiting eukaryotic protein biosynthesis was determined. The target molecules (2 and 6) were thus incubated with the reticulocyte

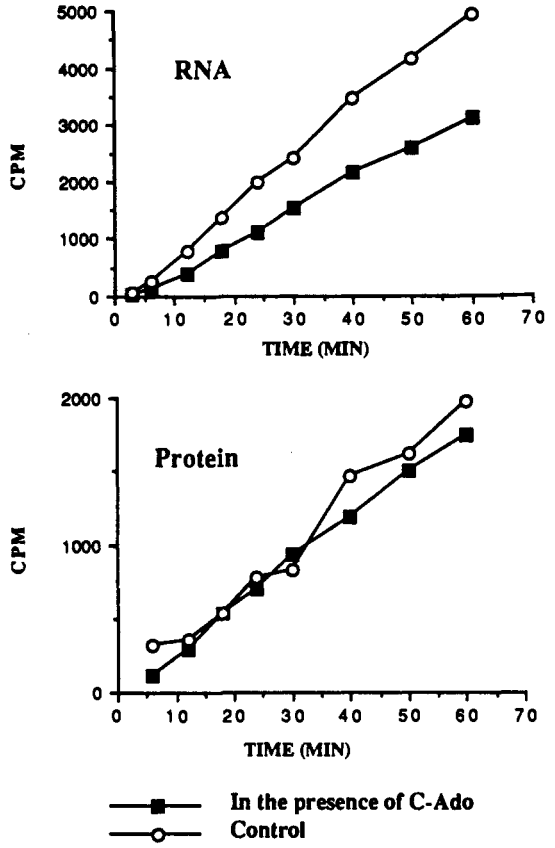


Figure 3. Biosynthesis of RNA and protein in P388 mouse leukemia cells in the presence of 90 μM concentration of C-Ado.

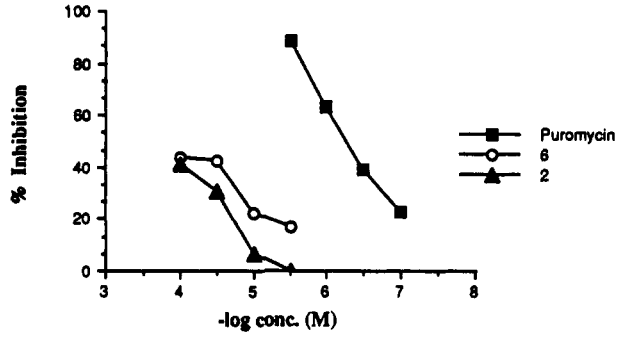


Figure 4. Percent inhibition of polyphenylalanine production in *E. coli* cell free system by agents **2**, **6**, and puromycin.

mixture using tobacco mosaic virus RNA as the messenger RNA and [^{14}C]leucine as the labeled amino acid. The initial results of this study are represented in Figure 6. Agents **2** and **6** were found to significantly inhibit (60–70%) protein biosynthesis at 3×10^{-5} M concentrations. Thus, it appears that these agents have a direct effect on protein biosynthesis. This effect appears to be greater (i.e. the agents are more active at lower concentrations) on eukaryotic protein biosynthesis than on prokaryotic protein biosynthesis.

Protein biosynthesis is not only the target of agents **2** and **6**, but it is also the target of many other 5'-sulfamoylated nucleosides. Nucleocidin (**1**) inhibits protein synthesis and, from studies of Florini, it appears that this compound forms a complex with the ribosome that somehow prevents translation; however, the precise nature of the nucleocidin–ribosome complex causing this to happen is unknown.[3a] 5'-Sulfamoyladenosine was found to slow protein synthesis in *E. coli* by inhibiting the formation of aminoacyl-tRNA's.[5] Ribavirin-5'-sulfamate

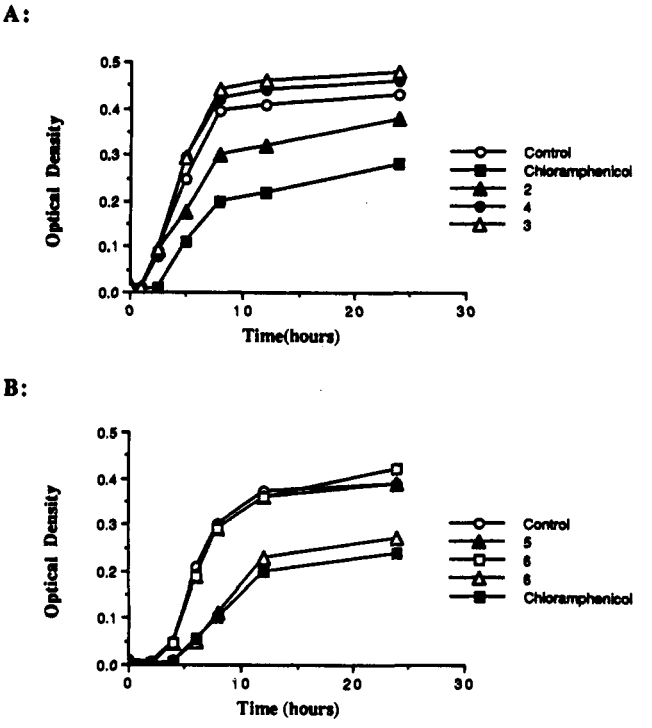


Figure 5. Growth of *E. Coli* B210 in the presence of (A) 10^{-4} M concentrations of **2**, **3**, and **4** and 3×10^{-6} M chloramphenicol, and (B) 2×20^{-4} M concentrations of **5**, **6**, and **8** and 3.3×10^{-6} M chloramphenicol.

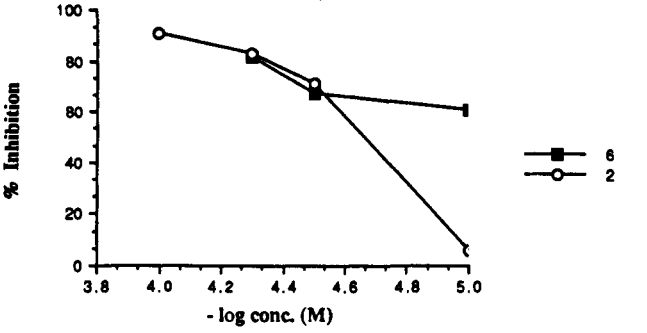


Figure 6. Percent inhibition of protein biosynthesis in rabbit reticulocyte lysate in the presence of agents **2** and **6**.

interferes with protein biosynthesis in rabbit reticulocyte lysate, also by inhibiting aminoacyl-tRNA formation.[10] It is possible that the new sulfamoylated carbocyclic derivatives (**2** and **6**) may also inhibit aminoacyl-tRNA synthetase. Further work will be necessary to determine the exact step in protein synthesis that these agents are acting.

Agent **2** was tested for substrate activity with adenosine deaminase and adenylic acid deaminase. It was found to be a poor substrate for adenosine deaminase and it was not a substrate for adenylic acid deaminase. Therefore, this agent should survive in vivo detoxification via deamination.

Conclusion

From this study of sulfamoylated carbocyclic nucleosides, two promising antitumor compounds, **2** and **6**, were discovered. The biological evaluation thus far suggests that compounds **2** and **6** are very toxic to eukaryotic, but not prokaryotic cells. These agents and compound **3** exhibited considerable activity against P388 tumor cell line; however, when the compounds were tested against *E. coli* B210 bacteria, toxicity was only seen at high con-

centrations. The other sulfamoylated agents studied (4 and 5) did not display any notable toxicity. These agents lacked the 6-amino group, and the results suggest that the amino group may be important for the cytotoxicity of these types of compounds. Also, agents 4 and 5 have greater steric bulk at the 6 position than do agents 2, 3, and 6. This extra bulk may not be easily tolerated at the still unknown site of action of these sulfamoylated antitumor agents. Furthermore, the results of mechanism of action studies using whole P388 cells and radioactive small molecules indicate that protein biosynthesis inhibition is a major target for agents 2 and 6. Also, the data from initial studies using a eukaryotic cell free protein biosynthesis system (rabbit reticulocyte lysate) support the claim that agents 2 and 6 have a direct effect on eukaryotic protein biosynthesis. Finally, the resistance of 2 to deamination by ADA and AMP deaminase will help this agent survive potential in vivo deactivation by these enzymes.

Experimental Section

Chemistry. Most of the reagents used were purchased commercially. If any additional drying or purification of these reagents was needed, the procedures are indicated in the individual experimental sections. Sulfamoyl chloride was prepared by a modification of the method of Graf;[28] however, this reagent was never isolated in pure form since it readily decomposes to hydrogen chloride and sulfamic acid in moist air.[28] The IR spectrum of the sulfamoyl chloride produced in this manner compared well with that reported[29] and was considered suitable for synthetic purposes. Unless otherwise indicated, for all solvent evaporations, removals, concentrations, or reductions in volume, a rotary evaporator which was attached to a water aspirator was used. All high-vacuum dryings and evaporations were done on a Welch Duo Seal 1400 vacuum pump. Chromatographic purification of products was performed using Merck Silica Gel 60 (230–400 mesh ASTM) in a flash chromatographic system. The product mixture was adsorbed onto the silica gel by dissolving the mixture in a solvent, adding a small amount of silica gel, evaporating the solvent on a rotary evaporator, and drying the remaining silica sample under high vacuum. The columns were packed using the more nonpolar of the eluting solvents. The eluting solvents are indicated in the individual experimentals. Thin-layer chromatography (TLC) was used to identify the product eluted from the column. Merck Silica Gel 60-F254 (0.25-mm precoated on glass plates) was used for TLC. The plates were developed by placing them in an iodine atmosphere and/or observing them under shortwave UV light. Once pure, all products were dried under high vacuum, usually in the presence of P_2O_5 to aid in the removal of water. The products were further characterized by their melting points (mp); their ultraviolet/visible (UV), infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and mass (MS) spectra; and their elemental analyses (EA). Melting points were determined on a Mel-Temp apparatus and are uncorrected. UV spectra were taken on a Beckman DU-70 spectrophotometer. IR spectra were taken on a Perkin-Elmer 281 or Nicolet 5DXC instrument. All compounds were in KBr pellet form for the IR analysis unless otherwise indicated. ^{1}H-NMR spectra were taken on a JEOL FX-90Q FT (89.55 MHz), a Nicolet NT-300 (300.0745 MHz), or a Bruker 200 (200.133 MHz) nuclear magnetic resonance spectrometer. However, unless otherwise indicated, all the reported spectra were taken on the Nicolet NT-300. The chemical shifts in the ^{1}H-NMR spectra are reported as parts per million (δ) relative to Me_2SO-d_6 (δ = 2.5 ppm). The ^{1}H-NMR data are presented as peak multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, qu = quintuplet, m = multiplet), coupling constant (J, reported in hertz), integration (no. of hydrogens), and signal assignment. See Scheme III for the general atom numbering for the new agents. Mass spectral data were recorded on an AEI MS-30 for electron impact (EI) type spectra and on a VG 7070E-HF model for fast atom bombardment (FAB) type spectra. In the EI spectral data, relative intensities are given in parentheses after the m/e value, with the base peak having a value of 100. If known, the fragments corresponding to other peaks are indicated after the relative intensity. The abbreviations used: M = mass peak and P = purine. The matrix and detection mode (+ or −) for FAB spectra are indicated. Elemental analyses were obtained from MHW Laboratories, Phoenix, AZ.

General Procedure for Acetonidation.[23,30] Compound 11, 15, or 17 was combined with a solution of p-toluenesulfonic acid monohydrate (p-TsOH·H_2O) in acetone (This solution had been dried overnight over 4-Å molecular sieves.) and with 2,2-dimethoxypropane (2,2-DMP). The solid slowly dissolved, and the solution was stirred at room temperature under nitrogen for 24–48 h. Saturated sodium bicarbonate (saturated $NaHCO_3$) solution was then added to the acetone solution and a white precipitate formed. The mixture was reduced in volume to an off-white solid. This solid was triturated with chloroform, and the triturate was reduced to a light brown or off-white solid identified as 12, 16, or 18.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-chloropurine (12). (±)-9-[β-(2α,3α-Dihydroxy-4β-(hydroxymethyl)cyclopentyl)]-6-chloropurine[24] (11, 0.5 g, 1.76 mmol), p-TsOH·H_2O (6.40 g, 33.6 mmol), acetone (210 mL), 2,2-DMP (15 mL), and saturated $NaHCO_3$ solution (250 mL) were used. Recrystallization from absolute ethanol provided a light brown solid which was identified as 12: total yield 0.48 g, 83%; mp 183–184 °C; R_f = 0.72 (chloroform–methanol 3:1); UV_{max} 265, 202 (0.1 N HCl); 265, 198 (H_2O); 265, 215 (0.1 N, NaOH); IR (cm^{-1}) 3323 (broad, O—H), 3067, 2941, 2902, 2894, [1593, 1557] C=C, C=N), 1382 ($C(CH_3)_2$), 1338, 1201, 1058; ^{1}H-NMR (Me_2SO-d_6) δ 8.84, 8.78 (each s, each 1 H, C2 and C8 H's), 5.12 to 4.87 (overlapping m's, 2 H, C1′H, C2′H), 4.81 (t, J = 5.2, 1 H, C5′OH), 4.56 (m, 1 H, C3′H), 3.51 (t, J = 5.0, 2 H, C5′H_2), 2.43 to 2.11 (m, 3 H, C4′H, C6′H_2), 1.48, 1.22 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); signal at 4.81 is D_2O exchangeable; MS (EI, 30 eV, 220 °C) m/e 325 (2.5, M^+), 311 (14.7), 309 (38.0), 266 (23.2), 237 (36.3), 236 (15.5), 235 (99.5), 219 (14.2), 180 (19.8), 155 (100, PH_2^+), 153 (56.8, P^+), 81 (59.2), 43 (71.1). Anal. ($C_{14}H_{17}N_4O_3Cl$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-(methylthio)purine (16). (±)-9-[β-(2α,3α-Dihydroxy-4β-(hydroxymethyl)cyclopentyl)]-6-(methylthio)-purine[23] (15, 1.10 g, 3.72 mmol), p-TsOH·H_2O (4.0 g, 21 mmol), acetone (200 mL), 2,2-DMP (20 mL), and saturated $NaHCO_3$ solution (170 mL) were used. The triturate was reduced to an off-white foamy solid which was identified as chromatographically pure 16. Total yield: 1.18 g, 95%. An analytical sample was prepared by recrystallization from ethyl acetate: mp of 124–126 °C; R_f = 0.72 (chloroform–methanol 3:1), R_f = 0.77 (concentrated ammonia–propanol 1:4); UV_{max} 294, 221, 204 (0.1 N HCl); 286, 221, 198 (H_2O); 293 (sh), 285, 222 (0.1 N NaOH); IR (cm^{-1}) 3351 (O—H), [3095, 2994, 2935, 2897] (C—H), 1569 (C=C, C=N), 1409, 1381 ($C(CH_3)_2$), 1337, 1206, 1064; ^{1}H-NMR (Me_2SO-d_6) δ 8.74, 8.60 (each s, each 1 H, C8 and C2 H's), 5.02 (m (skewed t), 1 H, C1′H), 4.91 (m, 1 H, C2′H), 4.80 (t, J = 5.3, 1 H, C5′OH), 4.57 (m, 1 H, C3′H), 3.52 (m, 2 H, C5′H_2), 2.67 (s, 3 H, C6-SCH_3), 2.40 to 2.14 (m, 3 H, C6′H_2, C4′H), 1.49, 1.23 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$): MS (EI, 30 eV, 220 °C) m/e 337 (3.3), 336 (8.8, M^+), 321 (7.0), 278 (12.8), 249 (10.8), 248 (14.3), 247 (100), 167 (40.7, PH_2^+), 166 (23.8, PH^+), 165 (10.5, P^+), 81 (19.3), 43 (19.8). Anal. ($C_{15}H_{20}N_4O_3S$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-amino-8-azapurine (18). (±)-9-[β-(2α,3α-Dihydroxy-4β-(hydroxymethyl)cyclopentyl)]-6-amino-8-azapurine[25] (17, 0.3 g, 1.13 mmol), p-TsOH·H_2O (4.96 g, 26.1 mmol), acetone (200 mL), 2,2-dimethoxypropane (20 mL), and saturated $NaHCO_3$ solution (150 mL). Recrystallization from absolute ethanol provided a white solid which was identified as pure 18: total yield 0.222 g, 64%; mp 230–232 °C; R_f = 0.66 (chloroform–

(28) Graf, R. *Chem. Ber.* **1959**, *92*, 509–513.
(29) Pouchert, C. J. *The Aldrich Library of Infrared Spectra*, 3rd ed.; Aldrich Chemical Co.: Milwaukee, 1981; p 542.
(30) Hampton, A. Nucleotides. II. A New Procedure for the Conversion of Ribonucleosides to 2′,3′-*O*-Isopropylidene Derivatives. *J. Am. Chem. Soc.* **1961**, *83*, 3640–3645.

methanol 3:1); UV_{max} 264, 207 (0.1 N HCl); 282, 204 (sh) (H_2O); 280, 216 (0.1 N NaOH); IR (cm^{-1}) 3466, 3283, 3088, 2917 (broad, O—H, N—H, C—H); [1691, 1614, 1571] (C=N, C=C, N=N), 1376 ($C(CH_3)_2$), 1325, 1216, 1070; ^{1}H-NMR (Me_2SO-d_6) δ 8.45, 8.11 (each bs, each 1 H, C6-NH_aH_b), 8.30 (s, 1 H, C2-*H*), 5.27 to 4.98 (overlapping m's, 2 H, C1'-*H*, C2'-*H*), 4.80 (t, *J* = 5.3, 1 H, C5'O*H*), 4.63 (m, 1 H, C3'*H*), 3.50 (t, *J* = 5.1, 2 H, C5'H_2), 2.46 to 2.18 (m, 3 H, C6'H_2, C4'*H*), 1.48, 1.24 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); signals at 8.45, 8.11, and 4.80 are D_2O exchangeable; MS (FAB, thioglycerol) 307 [(M + H)$^+$], 305 [(M − H)$^-$]. Anal. ($C_{13}H_{16}N_6O_3$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-(methylamino)purine (13). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-chloropurine (12, 0.64 g, 1.98 mmol) was combined with 40% methylamine in water (100 mL) in a stainless steel bomb. The bomb was sealed and heated to 85 °C for 48 h. The bomb was then cooled and opened. The reaction solution was reduced to an off-white solid (0.845 g). The solid was partitioned between water (50 mL) and chloroform (3 × 130 mL). The organic layers were combined and reduced to an off-white solid (0.625 g). Recrystallization from ethyl acetate–ether provided a white solid which was identified as **13**: total yield 0.519 g, 82%; mp 146–147 °C; R_f = 0.63 (chloroform–methanol 3:1): UV_{max} 263, 210 (0.1 N HCl); 266, 209 (H_2O); 266, 218 (0.1 N NaOH); IR (cm^{-1}) 3283 (broad, O—H, N—H), 2983, 2937, [1631, 1508] (C=C, C=N), 1381 ($C(CH_3)_2$), 1325, 1234, 1208, 1160, 1063; ^{1}H-NMR (Me_2SO-d_6) δ 8.24, 8.21 (each s, each 1 H, C2 and C8*H*'s), 7.66 (bs, 1 H, ArN*H*(CH_3)), 5.01 (t, *J* = 6.7, 1 H, C1'*H*), 4.91 to 4.69 (overlapping m's, 2 H, C5'O*H*, C2'*H*), 4.54 (m, 1 H, C3'*H*), 3.50 (s, 2 H, C5'H_2), 2.95 (bs, 3 H, NH(CH_3)), 2.23 (m, 3 H, C4'*H*, C6'H_2), 1.47, 1.22 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); signal at 7.66 disappears and signal at 4.91 to 4.69 simplifies in D_2O; MS (EI, 30 eV, 220 °C) *m/e* 319 (5.8, M$^+$), 304 (5.4), 261 (5.4), 231 (15.1), 230 (100), 205 (6.1), 204 (9.4), 176 (9.9), 150 (30.8, PH_2^+), 149 (15.2, PH^+), 120 (12.8), 81 (12.5), 43 (9.8). Anal. ($C_{15}H_{21}N_5O_3$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-(dimethylamino)purine (14). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-chloropurine (12, 0.643 g, 1.98 mmol) was combined with 40% dimethylamine in water (120 mL) in a stainless steel bomb. The bomb was sealed and heated to 100 °C for 18 h. The bomb was cooled and opened, and the reaction solution was reduced in volume to an off-white oily residue. This residue was partitioned between water (50 mL) and chloroform (3 × 150 mL). The organic layers were combined and reduced to a white solid (0.639 g). Recrystallization from ethyl acetate–methanol furnished a white solid which was identified as **14**: total yield 0.592 g, 90%; mp 164–165 °C; R_f = 0.74 (chloroform–methanol 3:1); UV_{max} 268, 213 (0.1 N HCl); 276, 214, 192 (H_2O); 276, 218 (0.1 N NaOH); IR (cm^{-1}) [3232, 3089] (O—H), 2916, 2865, [1605, 1558] (C=C, C=N), 1425, 1351 ($C(CH_3)_2$), 1299, 1074; ^{1}H-NMR (Me_2SO-d_6) δ 8.28, 8.21 (each s, each 1 H, C2 and C8*H*'s), 5.01 (t, *J* = 6.7, 1 H, C1'*H*), 4.94 to 4.69 (overlapping m's, 2 H, C5'O*H*, C2'*H*), 4.55 (m, 1 H, C3'*H*), 3.50 (m, 2 H, C5'H_2), 3.45 (s, 6 H, ArN(CH_3)$_2$), 2.23 (m, 3 H, C4'*H*, C6'H_2), 1.48, 1.23 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); Signal at 4.94–4.69 shrinks in size in D_2O; MS (EI, 30 eV, 150 °C) *m/e* 333 (7.5, M$^+$), 318 (6.1), 275 (5.3), 245 (16.3), 244 (100), 219 (6.9), 218 (7.6), 190 (8.3), 164 (20.0, PH_2^+), 134 (22.5), 43 (12.4). Anal. ($C_{16}H_{23}N_5O_3$) C, H, N.

Sulfamoyl Chloride.[28] Chlorosulfonyl isocyanate (6 mL, 67.6 mmol) was cooled to −24 °C in a dry ice–acetonitrile bath. As this cool liquid was stirred under nitrogen, concentrated hydrochloric acid (HCl, 1.8 mL (18.5 mmol HCl and 63.0 mmol H_2O)) was dripped in cautiously over 30 min (*vigorous* reaction) and a white precipitate formed. Once all the HCl was added, the mixture was warmed to 40 °C and it became a cloudy solution. After 30 min at 40 °C, the mixture was cooled to room temperature, combined with dichloromethane, and placed in the freezer. White crystals slowly formed in the cool mixture and, after standing overnight in the freezer, the mixture was further cooled to −42 °C and more crystals formed. The white crystals were isolated by filtration and weighed 5.49 g (69%, impure). The crystals were identified as impure sulfamoyl chloride (NH_2SO_2Cl) and were stored in the freezer for use in future reactions. [IR (neat, cm^{-1}) 3627, 3381, 3282, 1546, (1391, 1180, (S=O)), 1068, 927. These peaks agree with the published spectrum.[29]] The product gave off a sulfur-like odor at room temperature. The odor was presumed to be a result of the compound's decomposition to HCl and sulfamic acid. Therefore, because of this rapid decomposition, accurate values for the number of equivalents used in each reaction was not known; thus, only *approximate* weights are given in the reaction procedures.

General Procedure for Sulfamoylation. A suspension of compound **7**, **9**, **13**, **16**, or **18**, sodium hydride (NaH), and anhydrous ethylene glycol dimethyl ether (DME) was stirred under nitrogen at room temperature or 50–60 °C for 2 to 3 h. The suspension was cooled in an ice bath, and a solution of NH_2SO_2Cl in DME was added dropwise. The resulting mixture was stirred at 4 °C overnight. The excess NaH was then destroyed by adding water or methanol dropwise until no more effervescence could be seen. The mixture was reduced to a white-gray solid and this was dissolved in water and adsorbed onto silica gel. The silica sample was placed on a silica column, and the column was washed with ethyl acetate–methanol (10:1). Fractions containing product were combined and reduced in volume to solids **8**, **10**, and **19–21**.

9-[[2-(Sulfamoyloxy)ethoxy]methyl]adenine (8). 9-[(2-hydroxyethoxy)methyl]adenine[22] (**7**, 0.509 g, 2.4 mmol), NaH (0.400 g, 16 mmol), DME (50 mL), and NH_2SO_2Cl (1.1 g) in DME (10 mL) were used. **8** was isolated as a white solid: total yield 0.113 g, 27%; mp 166 °C dec; R_f − 0.31 (ethyl acetate–water:1-propanol 4:2:1); UV_{max} 257, 206 (0.1 N HCl); 259, 207 (H_2O); 259, 215 (0.1 N NaOH); IR (cm^{-1}) [3323, 3145] (N—H), 2953 (C—H), [1676, 1608] (C=N, C=C), [1357, 1176] (S=O), 1138, 1034, 922; ^{1}H-NMR (Me_2SO-d_6) δ 8.26, 8.16 (each s, each 1 H, C2 and C8 *H*'s), 7.47 (s, SO_2NH_2), 7.28 (s, 2 H, C6-NH_2), 5.57 (s, 2 H, C1'H_2), 4.09, 3.75 (each m, each 2 H, C2'H_2 and C3'H_2); signals at 7.47 and 7.28 are D_2O exchangeable; MS (FAB, thioglycerol) 289 [(M + H)$^+$]. Anal. ($C_8H_{12}N_6O_4S$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]adenine (10). Method A (±)-9-[β-(2α,3α-di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-adenine[23] (**9**, 0.596 g, 1.95 mmol), NaH (0.300 g, 12.5 mmol), DME (50 mL), NH_2SO_2Cl (0.650 g) in DME (30 mL). **10** was isolated as a white solid: total yield 0.382 g, 52%; mp 255 °C dec; R_f = 0.62 (ethyl acetate–methanol 1:1); UV_{max} 259, 212 (0.1 N HCl); 262, 206 (H_2O); 257, 218 (0.1 N NaOH); IR (cm^{-1}) [3492, 3347, 3309, 3145] (N—H), [2989, 2940] (C—H), [1669, 1598] (C=C, C=N), 1373 ($C(CH_3)_2$), 1208, 1180 (S=O), 1074; ^{1}H-NMR (Me_2SO-d_6) δ 8.26, 8.13 (each s, each 1 H, C2 and C8*H*'s), 7.52 (s, 2 H, H_2NSO_2), 7.21 (s, 2 H, C6-NH_2), 5.05 (m (skewed t), 1 H, C1'*H*), 4.84 (m, 1 H, C2'*H*), 4.58 (m, 1 H, C3'*H*), 4.12 (d, *J* = 6.3, 2 H, C5'H_2), 2.50 to 2.22 (bm, 3 H, C4'*H*, C6'H_2), 1.49, 1.24 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); Signals at 7.52 and 7.21 are D_2O exchangeable; MS (FAB; thioglycerol) 385 [(M + H)$^+$], 383 [(M − H)$^-$], 419 [(M + Cl)$^-$]. Anal. ($C_{14}H_{20}N_6O_5S$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(methylamino)purine (19). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-(methylamino)purine (**13**, 0.491 g, 1.54 mmol), NaH (~0.150 g, 6.25 mmol), DME (60 mL), and NH_2SO_2Cl (~0.450 g) in DME (30 mL) were used. Column fractions containing **19** were combined and reduced to a white solid (476 mg). Recrystallization from methanol–ether provided **19** as a white solid: total yield 0.290 g, 47%; mp 180 °C dec; R_f = 0.69 (ethyl acetate–methanol 1:1); UV_{max} 263, 210 (0.1 N HCl); 269, 209 (H_2O); 267, 216 (0.1 N NaOH); IR (cm^{-1}) [3382, 3217, 3000], (N—H), [1627, 1574] (C=C, C=N), 1390 to 1352 (S=O and $C(CH_3)_2$), 1323, 1176 (S=O), 1080; ^{1}H-NMR (Me_2SO-d_6) δ 8.25, 8.22 (each s, each 1 H, C2 and C8*H*'s), 7.70 (bs, 1 H, ArN*H*(CH_3)), 7.53 (s, 2 H, H_2NSO_2), 5.07 (m (skewed t), 1 H, C1'*H*), 4.85 (m, 1 H, C2'*H*), 4.60 (m (apparent q), 1 H, C3'*H*), 4.13 (d, *J* = 6.3, 2 H, C5'H_2), 2.97 (s, 3 H, NH(CH_3)), 2.50 to 2.23 (m, 3 H, C4'*H*, C6'H_2), 1.49, 1.24 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); signals at 7.70 and 7.53 are D_2O exchangeable; MS (FAB, thioglycerol 399 [(M + H)$^+$]. Anal. ($C_{15}H_{22}N_6O_5S$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(methylthio)purine (20). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-(methylthio)purine (**16**, 0.471 g, 1.4 mmol), NaH (~0.200 g, 8.3

mmol), DME (60 mL), and NH_2SO_2Cl (~0.400 g) in DME (30 mL) were used. **20** was identified as a white solid. Total yield: 0.384 g, 66%. Analytical samples were prepared by recrystallization from methanol–water (mp 215 °C dec) and from methanol (mp 155 °C dec): R_f = 0.79 (ethyl acetate–methanol 1:1); UV_{max} 295, 287 (sh), 222, 201 (0.1 N HCl); 292 (sh), 286, 220, 194 (H_2O); 286, 222 (0.1 N NaOH); IR (cm^{-1}) [3296, 3191, 3145] (N—H), 2988, 2909, 1572 (C=C, C=N), 1374 ($C(CH_3)_2$), 1209, 1179 (S=O), 1072; ^{1}H-NMR (Me_2SO-d_6) δ 8.74, 8.61 (each s, each 1 H, C8 and C2*H*'s), 7.54 (s, 2 H, H_2NSO_2), 5.07 (m, 1 H, C1′*H*), 4.96 (m, 1 H, C2′*H*), 4.61 (m, 1 H, C3′*H*), 4.15 (d, J = 6.0, 2 H, C5′H_2), 2.67 (s, 3 H, C6-SCH_3), 2.50 to 2.16 (m, 3 H, C6′H_2, C4′*H*), 1.51, 1.25 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); Signal at 7.54 is D_2O exchangeable; MS (FAB, thioglycerol) 416 [(M + H)$^+$], 414 [(M − H)$^-$], 450 [(M + Cl)$^-$]. Anal. ($C_{15}H_{21}N_5O_5S_2$) C, H, N.

(±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-amino-8-azapurine (21). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-amino-8-azapurine (**18**, 0.346 g, 1.13 mmol), NaH (~0.150 g, 6.25 mmol), DME (40 mL), and NH_2SO_2Cl (~0.300 g) in DME (20 mL) were used. Impure product was collected as a white solid (0.364 g). Recrystallization of this solid from methanol furnished pure **21**: total yield 0.274 g, 63%; mp 225 °C; R_f = 0.76 (ethyl acetate–methanol 1:1); UV_{max} 264, 207 (0.1 N HCl); 279, 203 (H_2O); 279, 216 (0.1 N NaOH): IR (cm^{-1}) [3357, 3296, 3109] (N—H), 1677, 1605, 1572] (C=C, C=N, N=N), 1374 ($C(CH_3)_2$), [1322, 1184] (S=O), 1077; ^{1}H-NMR (Me_2SO-d_6) δ 8.44, 8.11 (each bs, each 1 H, C6-NH_aH_b), 8.31 (s, 1 H, C2-*H*), 7.52 (s, 2 H, H_2NSO_2), 5.19 to 5.07 (overlapping m's, 2 H, C1′*H*, C2′*H*), 4.67 (m (apparent q), 1 H, C3′*H*), 4.15 (d, J = 5.7, 2 H, C5′H_2), 2.56 (m, 2 H), 2.38 (m, 1 H), [C6′H_2, C4′*H*], 1.51, 1.26 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$); signals at 8.44, 8.11, and 7.52 are D_2O exchangeable; MS (FAB: thioglycerol) 386 [(M + H)$^+$], 384 [(M − H)$^-$]. Anal. ($C_{13}H_{19}N_7O_5S$) C, H, N.

Method B Used To Make 10. (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]adenine (**9**, 0.399 g, 1.31 mmol) was combined with bis(tributyltin) oxide (2.3 g, 3.86 mmol) and anhydrous benzene (40 mL), and the mixture was refluxed under nitrogen for 2 h. The mixture, which became a solution while refluxing, was then cooled in an ice bath. NH_2SO_2Cl (0.450 g) dissolved in anhydrous dioxide (15 mL) was added dropwise to the cool solution. The resulting solution stirred at 4 °C overnight; however, the reaction was not complete at this time according to TLC; therefore, more NH_2SO_2Cl (0.500 g) in anhydrous dioxide (21 mL) was added, and the solution continued stirring overnight at 4 °C. The solvents were evaporated, and the remaining residue was neutralized with methanol–concentrated ammonia (1:1, 50 mL). The solvents were again evaporated and the residue that remained was dissolved in methanol and water and adsorbed onto silica gel (8 g). The silica sample was placed on a silica column (80 g), and the column was washed with ethyl acetate–methanol 10:1 (~800 mL). Fractions containing **10** were combined and reduced in volume to a white solid (0.342 g) which was identified as impure **10**. The solid was washed with *n*-pentane, and the white solid that remained was identified as pure **10**: total yield 0.170 g, 34%; mp 255 °C.

General Procedure for Removal of the Acetonide. Compound **10**, **19**, **20**, or **21** was dissolved in 90% aqueous trifluoroacetic acid (CF_3CO_2H/H_2O) and the solution was stirred at room temperature for 2 h. The solvent was then evaporated, and the remaining residue was dissolved in methanolic ammonia ($MeOH–NH_4OH$, 1:1). The solvent was again removed, and the remaining syrup was crystallized from water to provide **2**, **3**, **5**, and **6**.

(±)-9-[β-(2α,3α-Dihydroxy-4β-[(sulfamoyloxy)methyl]cyclopentyl)]adenine (2). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]adenine (**10**, 0.517 g, 1.35 mmol), $CF_3CO_2H–H_2O$ (30 mL) and $MeOH–NH_4OH$ (20 mL) were used. **2** was isolated as an off-white solid: total yield 0.302 g, 65%; mp 157–159 °C; R_f – 0.19 (ethyl acetate–water–propanol 4:2:1); UV_{max} 259, 211 (0.1 N HCl); 261, 205, 193 (H_2O); 261, 216 (0.1 N NaOH); IR (cm^{-1}) [3415, 3126 (broad)] (N—H, O—H), [1665, 1610, 1577] (C=N, C=C), 1354, 1186 (S=O); ^{1}H—NMR (Me_2SO-d_6) δ 8.18, 8.11 (each s, each 1 H, C2 and C8*H*'s), 7.49 (s, 2 H, H_2NSO_2), 7.18 (s, 2 H, C6-NH_2), 5.07 (d, J = 6.4, 1 H, C2′O*H*), 4.89 (d, J = 4.4, 1 H, C3′O*H*), 4.69 (m, 1 H, C1′*H*), 4.38 (m, 1 H, C2′*H*), 4.15, 4.07 (each m, each 1 H, C5′H_aH_b) 3.87 (m, 1 H, C3′*H*), 2.31 (m, 2 H), 1.78 (m, 1 H) [C6′H_2 and C4′*H*]; signals at 7.49, 7.18, 5.07, and 4.89 are D_2O exchangeable; MS (FAB; thioglycerol) 345 [(M + H)$^+$], 343 [(M − H)$^-$], 379 [(M + Cl)$^-$]. Anal. ($C_{11}H_{16}N_6O_5S$) C, H, N.

(±)-9-[β-(2α,3α-Dihydroxy-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(methylamino)purine (3). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(methylamino)purine (**19**, 0.253 g, 0.64 mmol), $CF_3CO_2H–H_2O$ (15 mL), and $MeOH/NH_4OH$ (10 mL) was used. **3** was used as a white solid: total yield 0.164 g, 72%; mp 178 °C dec, R_f = 0.35 (concentrated ammonia–propanol 1:4); UV_{max} 264, 211 (0.1 N HCl); 267, 209 (H_2O); 267, 218 (0.1 N NaOH); IR (cm^{-1}) [3446, 3273, 3150] (broad N—H, O—H), 1628 (C=C, C=N), [1362, 1186] (S=O); ^{1}H-NMR (Me_2SO-d_6) δ 8.19, 8.15 (each s, each 1 H, C2 and C8*H*'s), 7.62 (s, 1 H, ArN*H*(CH_3)), 7.47 (s, 2 H, H_2NSO_2), 5.05 (d, J = 6.4, 1 H, C2′O*H*), 4.87 (d, J = 4.4, 1 H, C3′O*H*), 4.70 (m, 1 H, C1′*H*), 4.37 (m, 1 H, C2′*H*), 4.15, 4.07 (each m, each 1 H, C5′H_aH_b), 3.87 (m, 1 H, C3′*H*), 2.96 (bs, 3 H, NH(CH_3)), 2.30 (m, 2 H), 1.80 (m, 1 H) [C6′H_2 and C4′*H*]; signals at 7.62, 7.47, 5.05, and 4.87 are D_2O exchangeable; MS (FAB, thioglycerol) 359 [(M + H)$^+$], 357 [(M − H)$^-$]. Anal. ($C_{12}H_{18}N_6O_5S$) C, H, N.

(±)-9-[β-(2α,3α-Dihydroxy-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(methylthio)purine (5). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(methylthio)purine (**20**, 0.373 g, 0.90 mmol), $CF_3CO_2H–H_2O$ (15 mL), and $MeOH/NH_4OH$ (10 mL) were used **5** was isolated as an off-white solid: total yield 0.207 g, 61%; mp 147–150 °C dec; R_f = 0.38 (concentrated ammonia–1-propanol 1:4); R_f = 0.59 (ethyl acetate–water–1-propanol 4:2:1); UV_{max} 295, 223, 202 (0.1 N HCl); 291, 286, 214, 193 (H_2O; 292, 286, 222 (0.1 N NaOH): IR (cm^{-1}) 3296 (broad, O—H), [1581, 1563] (C=C, C=N), [1359, 1184] (S=O); ^{1}H-NMR (Me_2SO-d_6) δ 8.73, 8.54 (each s, each 1 H, C8 and C2*H*'s), 7.52 (s, 2 H, H_2NSO_2), 5.13 (d, J = 6.3, 1 H, C2′O*H*), 4.96 (d, J = 4.3, 1 H, C3′O*H*), 4.82 (m, 1 H, C1′*H*), 4.43 (m, 1 H, C2′*H*), 4.13 (m, 2 H, C5′H_2), 3.90 (m, 1 H, C3′*H*), 2.68 (s, 3 H, C6-SCH_3), 2.33 (m, 2 H), 1.62 (m, 1 H) [C6′H_2, C4′*H*]; signals at 7.52, 5.13, and 4.96 are D_2O exchangeable; MS (FAB thioglycerol) 376 [(M + H)$^+$], 374 [(M − H)$^-$], 410 [(M + Cl)$^-$]. Anal. ($C_{12}H_{17}N_5O_5S_2$) C, H, N.

(±)-9-[β-(2α,3α-Dihydroxy-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-amino-8-azapurine (6). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-amino-8-azapurine (**21**, 0.251 g, 0.65 mmol), $CF_3CO_2H–H_2O$ (15 mL), and $MeOH–NH_4OH$ (10 mL) were used. **6** was isolated as a white solid: total yield 0.112 g, 50%; mp 179–180 °C; R_f = 0.35 (concentrated ammonia–propanol 1:4); UV_{max} 263, 207 (0.1 N HCl); 270, 204 (H_2O); 279, 216 (0.1 N NaOH); IR (cm^{-1}) 3162 (broad, O—H, N—H), [1700, 1617, 1573] (C=C, C=N, N=N), 1393, 1342, 1320, 1183 (S=O); ^{1}-NMR (Me_2SO-d_6) δ 8.31, 8.06 (each bs, each 1 H, C6-NH_aH_b), 8.28 (s, 1 H, C2-*H*), 7.49 (s, 2 H, H_2NSO_2), 5.16 (d, J = 6.1, 1 H, C2′O*H*), 5.08 (m, 1 H, C1′*H*), 4.96 (d, J = 4.9, 1 H, C3′O*H*), 4.40 (m, 1 H, C2′*H*), 4.18, 4.11 (each m, each 1 H, C5′H_aH_b; merges to qu in D_2O), 3.95 (m, 1 H, C3′*H*), 2.40 (m, 2 H), 1.90 (m, 1 H), [C6′H_2, C4′*H*); signals at 8.31, 8.06, 7.49, 5.16, and 4.96 are D_2O exchangeable; MS (FAB; thioglycerol) 346 [(M + H)$^+$]. Anal. ($C_{10}H_{15}N_7O_5S$) C, H, N.

(±)-9-[β-(2α,3α-Dihydroxy-4β-[(sulfamoyloxy)methyl]cyclopentyl)]-6-(dimethylamino)purine (4). (±)-9-[β-(2α,3α-Di-*O*-isopropylidene-4β-(hydroxymethyl)cyclopentyl)]-6-(dimethylamino)purine (**14**, 0.230 g, 0.69 mmol) was combined with anhydrous DME (38 mL) and anhydrous triethylamine (0.30 mL, 2.2 mmol). The clear solution that formed was stirred at room temperature under nitrogen for 30 min and was then cooled in an ice bath. A solution of NH_2SO_2Cl (~0.300 g) dissolved in DME (15 mL) was added dropwise to the cool solution and a white precipitate formed. The mixture continued to stir for 1–1.5 h in the ice bath. The mixture was then warmed to room temperature and a solution formed. This solution was adsorbed onto silica gel (1.2 g) and purified on a silica column (20 g). The column was washed with ethyl acetate (2 L), and fractions containing product were combined and reduced in volume to a white residue which was identified as impure **22** (0.342 g). [Attempts to purify this intermediate were unsuccessful. However, a ^{1}H-NMR spectrum (Me_2SO-d_6) of it, although filled with impurity signals, contained key signals at δ 8.28, 8.20 (each s,

each 1 H, C2 and C8H's], 7.52 (s, 2 H, H_2NSO_2), 1.48, 1.23 (each s, each 3 H, $C(CH_3)_a(CH_3)_b$) which are characteristic of intermediate 22. Also, MS (FAB thioglycerol) has a signal at 411 [(M − H)⁻].] The residue was dissolved in 90% aqueous trifluoroacetic acid (15 mL), and the solution was stirred at room temperature for 2 h. The solution was reduced in volume to a white residue, and this was dissolved in methanolic ammonia (1:1, 10 mL). The solvent was evaporated from the basic solution, and a clear syrup remained. Crystallization of this syrup from water provided a white solid which was identified as 4: total yield from 14, 0.085 g, 33%; mp 124–126 °C; R_f = 0.36 (concentrated ammonia–propanol 1:4); UV_{max} 269, 213 (0.1 N HCl); 277, 213 (H_2O); 276, 219 (0.1 N NaOH); IR (cm^{-1}) [3177, 3114] (broad, O—H), [1608, 1565] (C=C, C=N), 1423, [1343, 1182] (S=O); ^1H NMR (Me_2SO-d_6) δ 8.19 (s, 2 H, C2 and C8H's), 7.49 (s, 2 H, H_2NSO_2), 5.05 (d, J = 6.5, 1 H, C2'OH), 4.89 (d, J = 4.5, 1 H, C3'OH), 4.72 (m, 1 H, C1'H), 4.36 (m, 1 H, C2'H), 4.16, 4.07 (each m, each 1 H, C5'H_aH_b), 3.87 (m, 1 H, C3'H), 3.45 (s, 6 H, $ArN(CH_3)_2$), 2.32 (m, 2 H), 1.74 (m, 1 H) [C6'H_2 and C4'H)]; signals at 7.49, 5.05, and 4.89 are D_2O exchangeable; MS (FAB, thioglycerol) 373 [(M + H)⁺]. Anal. ($C_{13}H_{20}N_6O_5S$) C, H, N.

Biology. The Fischers media, horse serum, and Earl's balanced salt solution were purchased from GIBCO, Inc. An RPMI 1640 amino acid kit was used to prepare the modified media. The [^{14}C]-2-uridine (56.8 mCi/mmol), [^{14}C]-L-leucine (303 mCi/mmol), and [^{14}C]-L-lysine (306 mCi/mmol) were obtained from New England Nuclear, Inc. The [^{14}C]thymidine (51.4 mCi/mmol) was purchased from Amersham, and the [^{14}C]-L-phenylalanine (405 mCi/mmol) was purchased from ICN. Centrifugation was done on a Sorvall RC2B instrument. Liquid scintillation was purchased using a Beckman LS 6800 counter. HA type filters were purchased from Millipore Corp. Ecolume liquid scintillation fluid was purchased from ICN. The *E. coli* paste used to prepare the ribosomes was purchased from Grain Processing Corp., Muscatine, IA. *E. coli* B210 was obtained from the U.S. Dept of Agriculture. The bacterial nutrient broth was purchased from Difco. The optical densities in the bacterial study were determined on a Bausch and Lomb (Spectronic 20) spectrophotometer. Buffer A contains 0.05 M potassium chloride, 0.01 M magnesium acetate, and 0.01 M Tris acetate (pH 7.5). Adenosine deaminase and adenylic acid deaminase were purchased from Sigma, Inc. A Beckman Model 25 UV spectrophotometer was used to evaluate the deaminase results.

Antitumor Assay. The compounds in Table I were evaluated as in vitro antitumor agents by using the protocol of the National Cancer Insitute.[27] The methodology used was described in ref 31. P388 Mouse Leukemia cells grown in Fishers media supplemented with 10% horse serum were incubated with and without (control) the compounds under study at various concentrations. The cells had a starting concentration of ~10^5 cells/mL and were incubated at 37 °C for 3 days. Cell growth was determined with a hemacytometer, and puromycin was used as a positive control. The approximate concentration of each test compound which inhibits cell growth by 50% (IC_{50}) is reported in Table I.

Assay for DNA, RNA, and Protein Biosyntheses. A modified Fishers media was prepared which contained only half the recommended concentrations of L-phenylalanine (Phe, 30 mg/L used), L-leucine (Leu, 15 mg/L used), and L-lysine (lys, 25 mg/L used). For the protein assay, 10 λ each of [^{14}C]Phe, [^{14}C]-Leu, and [^{14}C]Lys were added to 11 mL of the modified media. One milliliter of this radioactive solution was then removed for the background counts. The remaining 10 mL was separated into two 5-mL portions. To one of these portions, the compound to be tested (2 or 6) was added (test). The other portion would serve as the control. Both portions were then combined with 5 mL of P388 cells suspended in the modified media to give a final cell concentration of 10^6 cells/mL. The 10-mL portions were incubated at 37 °C (water bath) and every 6–10 min a 1-mL fraction was removed from each portion and added to 1 mL of chilled (ice bath) Earl's balanced salt solution (EBSS). Each fraction was then treated as follows. The EBSS solution was centrifuged for 5 min at 270*g* to pellet the cells. The supernatant was then decanted, and the remaining cell pellet was resuspended in 2 mL of EBSS and centrifuged as before. The supernatant was again decanted and the pellet suspended in 1 mL of water to lyse the cells. The water mixture was placed in an ice bath for 1 h, and 2 mL of cold 10% trichloroacetic acid (TCA) was added to precipitate the macromolecules. The acidic mixture was kept in an ice bath for 15 min, and then the mixture was filtered through a Millipore type HA buffer (0.45 μm). The filter was washed with 3 × 2 mL of ice cold 5% TCA, and then the filter was dried under a heat lamp for 30 min. The dry filter was suspended in 10 mL of Ecolume (ICN), and radioactivity was counted via liquid scintillation. The number of counts reflects the amount of radioactive amino acuid incorporation into precipitated protein. Thus, the number of counts is proportional to the amount of protein synthesis taking place.

A very similar assay was used for the RNA and DNA studies. The only difference was that the [^{14}C]amino acids were replaced with [^{14}C]-2-uridine for the RNA assay and [^{14}C]thymidine for the DNA assay. The results of the studies are depicted in Figures 1, 2, and 3.

Polycytidylic-Uridylic Acid-Directed Synthesis of Polyphenylalanine. The *E. coli* protein biosynthesis assays were essentially as described previously.[32,33] Compounds 2 and 6 and puromycin were dissolved in water containing 10% DMSO. In a series of test tubes chilled on ice, solutions of one agent (test) or water (control) were combined with cell-free protein biosynthesis components[32,33] devoid of the messenger RNA and also containing [^{14}C]Phe. Various concentrations of the test compounds were prepared. Protein biosynthesis was initiated by adding polycytidylic-uridylic acid to each tube and then incubating the tubes at 37 °C (water bath) for 30 min. The protein synthesis was stopped by adding sodium hydroxide to the tubes and incubating for an additional 15 min at 37 °C. Hydrochloric acid was then added to neutralize the mixtures. The tubes were placed in an ice bath and combined with 10% TCA (2 mL per tube) to precipitate the proteins. The contents of the tubes were then filtered through Millipore type HA filters (0.45 μm pore size) and washed with ice cold 5% TCA (3 × 2 mL). The filters were dried under a heat lamp for 30 min, combined with 10 mL of Ecolume in scintillation vials, and counted with a liquid scintillation counter. Radioactive counts reflect [^{14}C]Phe incorporation into protein; thus, the number of counts is proportional to the amount of protein biosynthesis occurring. The percent inhibition of protein biosynthesis was determined for 2, 6, and puromycin at various concentrations by comparing the number of counts in the test and control vials. The results of this study was presented in Figure 4.

Antibacterial Assay. *E. coli* B210 bacteria were grown overnight in nutrient broth media at 32 °C in a shaking water bath. (Nutrient broth media contains 8 g of nutrient broth and 10 g of dextrose in a 1-L aqueous solution.) The compounds used for testing for antibacterial action and chloramphenicol, the positive control, were prepared as aqueous solutions containing 2% DMSO. These solutions were sterilized by passage through Millipore type HA filters (0.22-μm pore size). 2-fold serial dilutions of the agents in 1.5 mL of water were prepared in sterile tubes equipped with sterile cotton plugs. An inoculant of an overnight culture of bacterial was suspended in 2× nutrient broth media, and 1.5 of mL of this suspension was added to each tube in the series. The 3-mL mixtures in the tubes were incubated at 32 °C in a water bath. Bacterial cell growth was followed by increase in optical density at 465 nm using a Spectronic 20 spectrophotometer. The tubes were monitored for up to 24 h, and the results are presented in Figure 5.

Rabbit Reticulocyte Lysate Assay. A commercially available translation kit was purchased from Boehringer Mannheim. The kit contained nuclease-treated rabbit reticulocyte lysate which was depleted to amino acids; however, an amino acid

(31) Vince, R.; Daluge, S.; Brownell, J. Carbocyclic Puromycin: Synthesis and Inhibition of Protein Biosynthesis. *J. Med. Chem.* **1986**, *29*, 2400–2403.

(32) McFarlan, S. C.; Vince, R. Inhibition of Peptidyltransferase and Possible Mode of Action of a Dipeptidyl Chloramphenicol Analog. *Biochem. Biophys. Res. Comm.* **1984**, *122*, 748–754.

(33) Vince, R.; Daluge, S. Synthesis of Cyclohexyl Carbocyclic Puromycin and Its Inhibition of Protein Synthesis. *J. Med. Chem.* **1977**, *20*, 930.

solution was provided which was devoid of leucine. The standard assay procedure described in the kit was performed. Tobacco mosaic virus RNA was used as the messenger RNA at a final concentration of 1 μg/mL. [^{14}C]Leucine was used as the amino acid label. At the end of the 60-min incubation at 30 °C, the reaction mixture was terminated with sodium hydroxide and combined with 10% TCA (2 mL) to precipitate the protein. The mixture was filtered through a Millipore type HA filter (0.45 μm) and washed with ice-cold 5% TCA (4 × 3 mL). The filters were dried, and the protein was counted via liquid scintillation as described above. Various concentrations of agents **2** and **6** and cyclohexamide were incubated with the reticulocyte system. The percent inhibition of protein biosynthesis in the presence of these agents was determined and is depicted in Figure 6.

Adenosine Deaminase Assay. In the standard assay procedure, 1 mL of potassium phosphate buffer (0.05 M, pH 7.4) and calf intestinal mucosa adenosine deaminase (20 milliunits) were combined with adenosine or **2**. (Note: One unit of enzyme will deaminate one micromole of adenosine per minute at 25 °C at pH 7.5). The enzymatic reaction was run at 25 °C and the absorbance at 265 nm was followed for up to 10 min. A decrease in absorbance indicates that the reaction was occurring and thus, that a compound is a substrate for this enzyme. Agent **2** showed only a slight decrease in absorbance after 10 min and was therefore considered a slow substrate.

Adenylic Acid Deaminase Assay. In the standrd assay procedure, 1 mL of citric acid buffer (0.01 M, pH 6.5) and rabbit muscle adenylic acid deaminase (50 milliunits) were combined with AMP or **2**. (Note: One unit of enzyme will convert one micromole of 5′-AMP to 5′-IMP per minute at 25 °C at pH 6.5). The enzymatic reaction was run at 25 °C and absorbance at 265 nm was followed. A decrease in absorbance indicates that the reaction was occurring and, thus, that a compound is a substrate for this enzyme. No decrease in absorbance was seen when agent **2** was incubated with the enzyme; therefore, this agent was not a substrate.

Acknowledgment. We gratefully acknowledge the support of this work by the National Cancer Institute, Grant CA23263.