

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

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Bogotá, D.C.,

Abogado
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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ASUNTO	Radicación	09-22669	1 3986
	Trámite	011	
	Evento	378	
	Actuación	519	
	Oficio		
	Folios	008	

Patente de Invención	☐	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☐	Cap. I ☐	Cap. II ☐

No. Solicitud Internacional PCT/US2007/017463
Fecha de Presentación Internacional 06 de agosto de 2007.

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 114 a 264	05/marzo/2009
	Folios 336 a 524	07/junio/2009
Reivindicaciones:	Folios 265 a 274	05/marzo/2009
	Folios 532 a 548	11/octubre/2010
Prioridad:	US 60836158	08/agosto/2006

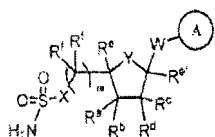
2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque: esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Compuesto de heteroarilo útiles como inhibidores de enzimas de activación"

El problema planteado en esta solicitud consiste en suministrar proporcionar compuestos útiles para desórdenes de proliferación celular como cáncer y



Compuesto I

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 31/505, A61P 35/00 y C07D 213/82 // 239/48 // 251/18 // 403/04 // 403/08 // 473/32 // 498/04

4. Excepción a la Patentabilidad (Art 20 D 486 literales (a) – (d))

El objeto de las reivindicaciones 25-27 no es patentable de acuerdo con el Art citado.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 – 41 no son concisas porque de las definiciones de R^a, R^b, R^c, R^d, R^e, R^f, R^g, R^h, R^k, W, D, E, F, G, N, Q, U, X, Y, Z, V¹, T¹, R¹, R^m, Rⁿ, R^d, R³, R⁴, R⁵, R⁶, R⁷, R⁸, R^{4x}, R^{4y}, R^{5x}, R⁶, R^{5x}, R^{1g}, R^{2g}, R^{3a}, R^{3b}, R^{8p}, R^{8k}, R^p, R^{9a}, R^{9b}, R^{2f},  se obtiene un gran número de compuestos, muchos de los cuales no están ejemplificados en la forma de ejemplos de la invención dentro del capítulo descriptivo, se sugiere restringir la solicitud a una razonable generalización del tipo de compuestos que han sido sintetizados o probados, es decir, que se encuentran debidamente sustentados. El siguiente cuadro ejemplifica la afirmación anterior y presenta los sustituyentes que se encuentran soportados en la descripción.

Sustituyentes de acuerdo con la reivindicación 1 de la solicitud		Sustituyentes soportados en la descripción
R ^a y R ^c	hidrógeno, fluoro, CN, -N ₃ , -OR ⁵ , -N(R ⁴) ₂ , -NR ⁴ CO ₂ R ⁶ , -NR ⁴ C(O)R ⁵ , -C(O)N(R ⁴) ₂ , -C(O)R ⁵ , -OC(O)N(R ⁴) ₂ , -OC(O)R ⁵ , -OCO ₂ R ⁶ , o un C ₁₋₄ , alifático o C ₁₋₄ fluoroalifático opcionalmente sustituido con un grupo seleccionado de -OR ^{5m} , -N(R ^{4x})(R ^{4y}), -CO ₂ R ^{5m} , o -C(O)N(R ⁴)(R ^{4*}); o R ^a y R ^c junto forman un enlace;	Hidrógeno y Hidroxilo
R ^b y R ^d	hidrógeno, fluoro, C ₁₋₄ alifático o C ₁₋₄ fluoroalifático	Hidrógeno y Hidroxilo
R ^f	hidrógeno, fluoro, un C ₁₋₄ , alifático o C ₁₋₄ fluoroalifático o dos R ^f tomados en forma conjunta forman =O, dos R ^f tomados en forma conjunta con el átomo de carbono al que están unidos, forman un anillo carbocíclico de 3-6 miembros; o R ^f , tomado en forma conjunta con R ^e y los átomos de carbono intervinientes forma un espirociclo de 3-6 miembros	Hidrógeno
W	-CH ₂ -, -CHF-, -CF ₂ -, -CH(R ¹)-, -CF(R ¹)-, -NH-, -N(R ¹)-, -O-, -S-, o -NHC(O)-	-CH ₂ -, -O-, -NH- y -N(R ¹),
X	-CH ₂ -, -CHF-, -CF ₂ -, -NH- y -O-	-NH- y -O-
Y	-O-, -S- o -C(R ^m)(R ⁿ)	-C(R ^m)(R ⁿ)

La reivindicación 37 no es clara porque refiere ser dependiente de la reivindicación 40, sin embargo, la reivindicación 40 simultáneamente refiere ser dependiente de la reivindicación 37 .

Descripción (Art 28 D 486)

La Descripción no es clara ni concisa porque la utilización de las expresiones (fol. 341) “y otros desórdenes asociados con la actividad de E1” y que (Fol. 341) “poner como blanco enzimas de activación E1 provee una oportunidad único de interferir con un variedad de importantes vías bioquímicas para mantener la integridad de la división celular y de la señalización celular” refiriéndose a las posibles actividades farmacológicas del compuesto no permite que la persona del oficio normalmente versada en la materia identifique o restrinja el ámbito de aplicación de la invención.

La descripción no clara porque no permite a una persona versada en la materia identificar un único concepto inventivo, dado que como lo manifiesta el solicitante a través de sus realizaciones preferidas I-110 y I-138 (Fol. 388 y 392) estos compuestos son inhibidores de la enzima de activación E1 pero no comparten los elementos técnicos esenciales de la invención.

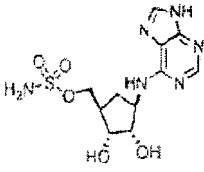
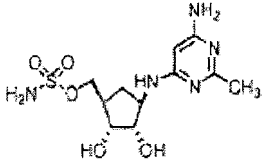
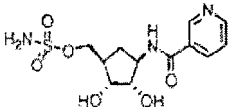
6. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el Requisito de Unidad de Invención porque se refiere a por lo menos 3, Grupos Inventivos de acuerdo con las realizaciones preferidas presentadas.

Los Grupos Inventivos están definidos por las siguientes características esenciales:

- 1° Compuestos en los cuales el núcleo está caracterizado por ser derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-[(9*H*-purin-6-amino ó oxo-il)ciclopentil]metilo.
- 2° Compuestos en los cuales el núcleo está caracterizado por ser derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-[(piridin ó pirimidin ó 1,3,5-triazina-((2 ó 4-amino ó oxo-il)ciclopentil]metilo
- 3° Compuestos en los cuales el núcleo está caracterizado por ser derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-{piridin-2-ilcarbonil)-amino}ciclopentil]metilo.

Los grupos Inventivos de acuerdo con las realizaciones preferidas, son descritos a continuación:

Grupo inventivo	Característica esencial de grupo inventivo (estructura química central)	Realizaciones preferida del grupo inventivo
1	 Compuesto I-3	3, 9, 34, 36-39, 41, 49, 56, 62-64, 67-70, 74, 80, 84, 87, 90, 93, 99-105, 107, 109,111,112, 117, 121-123, 126, 128-131, 137-140, 146 y 152.
2	 Compuesto I-13	1-2, 4-8, 10-15, 17-22, 24-33, 35, 40, 42-48, 50-55, 57-61, 65, 66, 71-73, 75-79, 81-83, 85, 86, 88, 89, 91, 92, 94-98,106,108,113-116,118-120,124-125,127,132-136,141-145,147-151 y 153.
3	 Compuesto I-16	16 y 23.

La evaluación de la unidad de invención para esta solicitud fue efectuada a través del método problema-solución en forma a posteriori y se presenta en el examen de patentabilidad efectuado al primer grupo inventivo, encontrándose que los documentos D1 y D2 anticipan el concepto común inventivo de la solicitud. Adicionalmente, se reitera que la solicitud presenta una estructura anular hipervariable del tipo A-B-C-D en lugar de una estructura central química definida. En consecuencia, la solicitud no cumple el requisito del Artículo 25 de la Decisión 486 de 2000 de la Comisión de la Comunidad Andina de Naciones. Este despacho sugiere presentar las solicitudes divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad agosto 8 de 2006 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	J. Med. Chem. 1992,35, 3991-4000	Synthesis and Biological Evaluation of 5'-Sulfamoylated Purinyl Carbocyclic Nucleosides	1992
D2	Bioorganic & Medicinal Chemistry Letters 15 (2005) 3389–3393	Deoxyribosyl analogues of methionyl and isoleucyl sulfamate adenylates as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases	2005

D1: Es un estudio que divulga (Abs.) los resultados de una serie de nucleósidos purinil carbocíclico 5'-sulfamoilados que fueron sintetizados y evaluados en su actividad antitumoral y antibacteriana.

Adicionalmente, divulga (Pág. 1, Col. 1) que muchos análogos de la nucleocidina defluorinados que contienen 5'sulfamoil grupo han sido evaluados y estos agentes muestran actividad antibacterial, antitumoral, antiviral, antiparasitaria y herbicida.

De igual modo, indica (Pág. 1, Col. 2) que cuando se sustituye el en anillo del azúcar del nucleósido, el átomo de Oxígeno por un radical metileno, se obtienen derivados de estructura muy similar a los naturales y que los productos mono, di o trifosforilados formados en estas reacciones son con frecuencia agentes antivirales y antitumorales.

También, Informa (Pág. 1, Col. 1) que todos los agentes seleccionados para este estudio contienen una adenina o un base similar a adenina y que esta selección fue basada en los resultados del estudio de estudio de Bloch y Coutsogeorgopoulos, donde también se incluían análogos defluorinados de la nucleocidina que contenían adenina, inosina, citidina, uracilo y timidina.

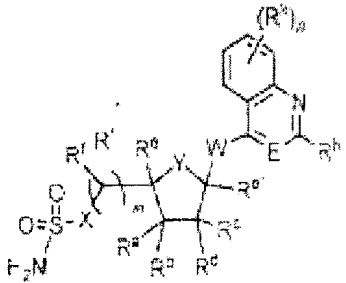
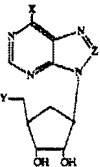
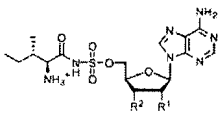
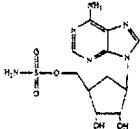
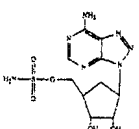
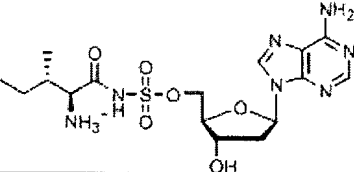
Por otra parte, da a conocer en la tabla 1 (Pág 3, Col. 2) que los resultados de los estudios de actividad de los compuestos, indicando que los compuestos 2 y 6 fueron agentes citotóxicos muy potentes y que los otros nuevos agentes 4 y 5, exhiben alguna toxicidad pero significativamente menor que la presentada por los compuestos 2 y 6.

modificaría el compuesto divulgado en D1 sustituyendo la adenina por un base similar a adenina, es decir un bioisótero ó análogos defluorinados de la nucleocidina que contengan adenina ó inosina ó citidina ó uracilo ó timidina de acuerdo con lo divulgado por D1, para así llegar al objeto de la invención. Por lo tanto la reivindicación 1 se considera obvia.

Dado que las reivindicaciones 2, 3, 5 y 15 a 17 no mencionan características técnicas esenciales, estás tampoco se puede considerar inventivas.

Teniendo en cuenta la falta de unidad de invención, se elige el primer grupo inventivo para estudiar la patentabilidad de la invención para la reivindicación 22 caracterizado por ser un compuesto derivados del sulfamato del [(1*R*,2*R*,3*R*,4*R*)-4-[(quinoleina ó quinazolina-6-amino ó oxo-il)ciclopentil]metilo.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2																		
Compuesto VII	El compuesto I	Una serie de compuesto de fórmula																		
 <table><tr><td>X</td><td>O</td></tr><tr><td>Y</td><td>CoO</td></tr><tr><td>W</td><td>NH2</td></tr><tr><td>E</td><td>NH2</td></tr><tr><td>R</td><td>H</td></tr><tr><td>R^K</td><td>H</td></tr></table>	X	O	Y	CoO	W	NH2	E	NH2	R	H	R ^K	H	 <table><tr><td>X</td><td>NH2</td></tr><tr><td>Z</td><td>C</td></tr><tr><td>Y</td><td>Un grupo Sulfamoi</td></tr></table>	X	NH2	Z	C	Y	Un grupo Sulfamoi	 Tle-AMP
X	O																			
Y	CoO																			
W	NH2																			
E	NH2																			
R	H																			
R ^K	H																			
X	NH2																			
Z	C																			
Y	Un grupo Sulfamoi																			
	El compuesto 2 y 6	El compuesto 2																		
	 compuesto 2  compuesto 6																			
	Indica que los agentes seleccionados para este estudio todos contienen una adenina o un base similar a adenina y que esta selección fue basada en los resultados del estudio de Bloch y Coutsogeorgopoulos, donde también se incluían análogos defluorinados de la nucleocidina que contenían adenina, inosina, citidina, uracilo y timidina.	Las modificaciones a este grupo de moléculas han estado enfocadas a tres regiones farmacofóricas que incluye los grupos adenina, ribosa y acil fosfato.																		

ciclopentil y no una ribosa como lo indica el documento D2

El efecto que logra el modificar una purina por una quinoleina ó quinazolina, es obtener nuevos compuesto inhibidores de la Enzima de activación E1 con actividad antitumoral.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo modificar los compuestos con actividad antitumoral descritos en D1 para así obtener compuesto alternativos inhibidores de la enzima de activación E1 y por lo tanto útiles en el tratamiento de enfermedades antiproliferativas.

Sin embargo, el documento D2 divulga que las modificaciones a este grupo de moléculas han estado enfocadas a tres regiones farmacofóricas que incluye los grupos adenina, ribosa y acil fosfato y el documento D1 muestra como la introducción de un grupo bioisostérico de la adenina como un derivado 3H-[1,2,3]triazolo[4,5-d]pirimidina no afecta la actividad actitumoral de los compuesto objeto de la invención.

En consecuencia, la persona del oficio normalmente versada en la materia modificaría el compuesto divulgado en D1 sustituyendo la adenina una serie bioisóterica como esta sugerido en el documento D2 de acuerdo con lo divulgado por D1, para así llegar al Objeto de la invención. Por lo tanto la reivindicación 22 se considera obvia.

9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	J. Med. Chem. 1992,35, 3991-4000	1-3, 5,15-17 y 22	Nivel inventivo
D2	Bioorganic & Medicinal Chemistry Letters 15 (2005) 3389-3393	22	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante (60 días) y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

18 AGO. 2012

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

Elaborado por: Lenin Roberto Guerrero. 
Revisado Por: Consuelo Leguizamón Leguizamón.
Aprobado por: José Luis Salazar López.

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

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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'-acetanide-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 *Escherichia 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 colossus*,^{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,^{5,6,7} antitumor,^{8,9} antiviral,^{10,11,12} antiparasitic,^{13,14} and herbicidal¹⁵ activities.



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.^{16,17} Carbocyclic adenosine (C-Ado), for example, forms a monophosphate capable of inhibiting at least one enzyme in purine de novo biosynthesis.¹⁷ This

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).^{19,20} 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.

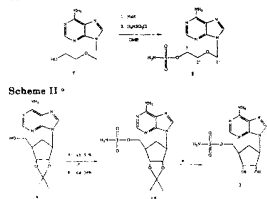
The carbocyclic agents chosen for this investigation all contain an adenine or adenine-like base. This choice was based on the results of a study by Bloch and Coutageorgopoulos²¹ where defluorinated analogs of nucleocidin containing adenine, inosine, cytidine, uracil, and thymine as the bases attached 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 8-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-azadenine base (6) was also attached to the carbocyclic ring since 8-azapurines have often exhibited greater antitumor activity than the natural purine agents.^{16,22} 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'-acetanide-protected carbocyclic nucleosides followed by deprotection. In the synthesis of 5'-sulfamoyladosine by Shuman et al.,²³ 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 acetamide-protected nucleoside by reacting with bis(triisopropyl)tin oxide and followed with the addition of sulfamoyl chloride.²⁴

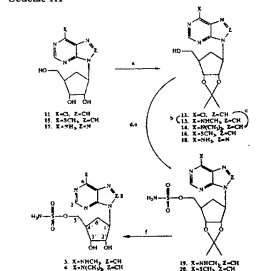
As a model reaction, acyclosadenosine (7) originally reported by Schaeffer et al.,²⁵ was reacted with sodium hydride and sulfamoyl chloride, and this produced 9-[(2-sulfamoyloxy)ethyl]methyladenosine (8) (Scheme I). Likewise, when 2',3'-isopropylidene carbocyclic adenosine

Scheme I



* (a) NaH, DME; (b) H_2NSO_2Cl , DME; (c) $(n-Bu)_3Sn_2O$, benzene; (d) H_2NSO_2Cl , dioxane; (e) CF_3CO_2H , H_2O .

Scheme II



(a) acetone, $p-TsOH$, 2,2-dimethoxypropane; (b) 40% NH_4OH in H_2O , 80 °C; (c) 40% NH_4OH , 100 °C; (d) NaH, DME; (e) H_2NSO_2Cl , DME; (f) CF_3CO_2H , H_2O .

(9), first reported by Sheely et al.,²⁶ was reacted under these conditions, intermediate 10 was isolated in a 52% yield (Scheme II). When 9 was activated with bis(triisopropyl)tin 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* 11, was protected as the acetamide to give 12 (Scheme III). The 6-chloro group in 12 was easily displaced with methanamine

(23) Sheely, T. F.; Clayton, J. D. Synthesis of Carbocyclic Analogues of Purine Ribonucleosides. *J. Am. Chem. Soc.* 1969, 91, 3073-3083.
(24) Sheely, T. F.; Clayton, J. D. Carbocyclic Analogs of 6-Substituted Purine Ribonucleosides and of Adenosine Ribonucleosides. *J. Pharm. Sci.* 1973, 62, 1252-1257.

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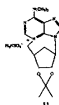
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and dimethylamine to produce intermediate 13 and 14, respectively. The 6-(thiomethyl) derivative was approached by first forming the 6-thiopurine from 6-chloropurine intermediate 12, followed by methylation. However, in one experiment, displacement 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 acetamide. 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.²⁴ The diol in 15 was protected with an acetamide to give 16. 8-Aza carboxylic adenosine²⁵ (17), was similarly protected to give acetamide 18.

Treatment of methylamino (13), methylthio (16), and 8-azadenosine (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, 4, and 6, respectively (Scheme III).

The dimethylamino derivative 4 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 N⁶,N⁶-dimethyl bases occur at δ 8.28 and δ 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 8.78²⁶. 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 35% 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.



Biological Results and Discussion

The compounds in Table I were evaluated for cytotoxicity against P388 mouse leukemia cells.²⁷ Puromycin, a

Table I. Cytotoxicity to P388 Mouse Leukemia Cells

	X	Z	Y	IC ₅₀ (μg/mL)
2	NH ₂	CH	OSO ₂ NH ₂	0.082–0.125
3	NHCH ₃	CH	OSO ₂ NH ₂	2–4
4	N(CH ₃) ₂	CH	OSO ₂ NH ₂	~74
6	SCH ₃	CH	OSO ₂ NH ₂	~50
8	NH ₂	N	OSO ₂ NH ₂	0.0166–0.0625
8 (acyclo)	NH ₂	CH	OSO ₂ NH ₂	>100
C-Ado	NH ₂	CH	OH	~0.5
17	NH ₂	N	OH	0.25–0.5
puromycin	—	—	—	0.25–0.5

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 (IC₅₀'s of 10^{-1} to 10^{-2} μg/mL). Agent 3 was also quite toxic, possessing an IC₅₀ 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. Carboxylic adenosine (C-Ado) and 8-aza carboxylic 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 derivative was not a result of hydrolysis of the 5'-sulfamoyl group and the release of C-Ado or 17.

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 [¹⁴C]uridine for RNA, [¹⁴C]thymidine for DNA, and [¹⁴C]-L-phenylalanine, [¹⁴C]-L-leucine and [¹⁴C]-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

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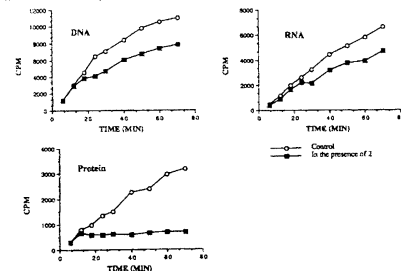


Figure 1. Biosynthesis of DNA, RNA, and protein in P388 mouse leukemia cells in the presence of 30 μM concentration of agent 2.

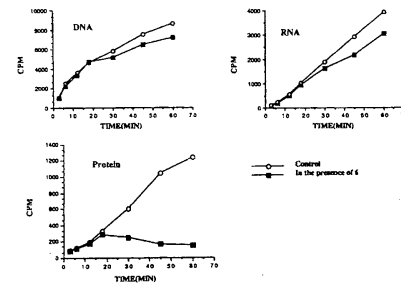


Figure 2. Biosynthesis of DNA, RNA, and protein in P388 mouse leukemia cells in the presence of 12 μM concentration of agent 6.

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^{-4} 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, was 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

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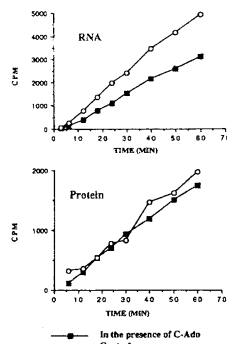


Figure 3. Biosynthesis of RNA and protein in P388 mouse leukemia cells in the presence of 90 μ M concentration of C-Ado.

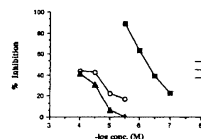


Figure 4. Percent inhibition of polyphenylalanine production in *E. coli* cell free system by agents 2, 6, and purtocyta.

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^{-6} 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.³⁴ 5'-Sulfamoyladenine was found to slow protein synthesis in *E. coli* by inhibiting the formation of aminoacyl-tRNA.³⁵ Ribavirin-5'-sulfamate

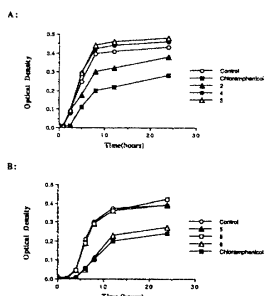


Figure 5. Growth of *E. coli* B210 in the presence of (A) 10^{-4} M concentrations of 2, 3, and 4 and 3×10^{-6} M chloramphenicol, and (B) 2×10^{-4} M concentrations of 3, 6, and 8 and 3.3×10^{-4} M chloramphenicol.

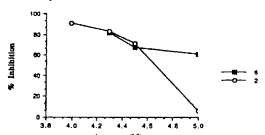


Figure 6. Percent inhibition of protein biosynthesis in rabbit reticulocyte lysate in the presence of agents 2 and 6.

interfere with protein biosynthesis in rabbit reticulocyte lysate, also by inhibiting aminoacyl-tRNA formation.³⁶ 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 detoxification 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 Graf³⁷ however, this reagent was never isolated in pure form since it readily decomposes to hydrogen chloride and sulfuric acid in moist air.³⁸ The IR spectrum of the sulfamoyl chloride produced in this manner compared well with that reported³⁸ 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 experimental sections. Thin-layer chromatography (TLC) was used to identify the product eluted from the column. Merck Silica Gel 60-F254 (0.25 mm precoat 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 (^1H -NMR) and mass (MS) spectra, and their elemental analyses (EA). Melting points were determined on a Met Temp apparatus and are uncorrected. UV spectra were taken on a Beckman DU-70 spectrophotometer. IR spectra were taken on a Perkin-Elmer 261 or Nicolet MDX instrument. All compounds were in KBr pellet form for the IR analysis unless otherwise indicated. ^1H -NMR spectra were taken on a JEOL FX-200 FT (200 MHz), a Nicolet NT-300 (300.13 MHz), or a Bruker AMX-300 (300 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 ^1H -NMR spectra are reported as parts per million (δ) relative to Me_4SiO_4 ($\delta = 2.5$ ppm). The ^1H -NMR data are presented as peak multiplicity (s = broad, d = doublet, t = triplet, q = quartet, qu = quintuplet, m = multiplet), coupling constant (J , reported in hertz), integration (no. of hydrogens), and signal assignment.

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See Scheme III for the general numbering for the new agents. Mass spectral data were recorded on an ARI MS-30 for electron impact (EI) type spectra and on a VG 7070E-HP model for fast atom bombardment (FAB) type spectra. In the EI spectra, 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 = parent. The matrix and detection mode (v/v) for FAB spectra are indicated. Elemental analyses were obtained from MHW Laboratories, Phoenix, AZ.

General Procedure for Acetonide Formation.³⁹ Compound 11, 15, or 17 was combined with a solution of p -toluenesulfonic acid monohydrate (p -TolOH-H₂O) 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 NaHCO₃) 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.

(4)-9-[8-(2a,3a-Di-O-isopropylidene-4 β -(hydroxymethyl)-cyclopentyl)-6-chloropurine] (12). (4)-9-[8-(2a,3a-Dihydroxy-4 β -(hydroxymethyl)cyclopentyl)-6-chloropurine] (1). 0.5 g (1.76 mmol), p -TolOH-H₂O (6.40 g, 33.6 mmol), acetone (210 mL), 2,2-DMP (15 mL), and saturated NaHCO₃ 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 181–184 °C; $R_f = 0.72$ (chloroform-methanol 3:1); UV (max 285, 290 (0.1 N HCl), 285, 188 (H₂O); 285, 215 (0.1 N NaOH); IR (cm⁻¹) 3332 (broad, O—H), 3067, 2941, 2902, 2884, [1699, 1567] C=C, C=N), 1382 (C(CH₃)₂), 1338, 1261, 1052; ^1H -NMR (Me₂SO- d_6) δ 8.84, 8.78 (each, 1 H, C2 and C8 H's), 5.12 (s, 4.87 (overlapping m, 2 H, C1', C2'), 4.81 (t, $J = 5.2$, 1 H, C5'OH), 4.56 (m, 1 H, C3' H), 3.11 (t, $J = 5.0$, 2 H, C3' H), 2.43 to 2.11 (m, 3 H, C4' H, C6' H), 1.48, 1.22 (each, 3 H, C(CH₃)₂(C₂H₅)) signals at 4.81 s [D₂O exchangeable]; MS (EI, 30 eV, 220 °C) m/e 335 (2.5, M⁺), 311 (14.7), 309 (38.0), 298 (23.3), 237 (36.3), 236 (15.5), 235 (99.5), 219 (14.2), 180 (18.8), 155 (100, P₂H₅), 153 (56.8, P₂H₅), 81 (59.2), 43 (71.1). Anal. (C₂₄H₂₄N₄O₆Cl₂) C, H, N, (4)-9-[8-(2a,3a-Di-O-isopropylidene-4 β -(hydroxymethyl)-cyclopentyl)-6-(methoxymethyl)purine] (16). (4)-9-[8-(2a,3a-Dihydroxy-4 β -(hydroxymethyl)cyclopentyl)-6-(methoxymethyl)purine] (1). 1.0 g (3.72 mmol), p -TolOH-H₂O (4.0 g, 21 mmol), acetone (200 mL), 2,2-DMP (20 mL), and saturated NaHCO₃ 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, 86%. An analytical sample was prepared by recrystallization from ethyl acetate: mp of 134–135 °C; $R_f = 0.72$ (chloroform-methanol 3:1); $R_f = 0.77$ (concentrated ammonia-propanol 1:4); UV (max 254, 221, 204 (0.1 N HCl); 286, 221, 188 (H₂O); 285 (ab), 285, 222 (0.1 N NaOH); IR (cm⁻¹) 3381 (O—H), 3055, 2994, 2935, 2897 (C—H), 1589 (C=C), C=N), 1408, 1381 (C(CH₃)₂), 1337, 1286, 1064; ^1H -NMR (Me₂SO- d_6) δ 8.74, 8.60 (each, 1 H, C8 and C2' H's), 5.02 (m, (overlapped), 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, C3' H's), 2.67 (s, 3 H, C6-SOCH₃), 2.40 to 2.14 (m, 3 H, C6' H, C4' H), 1.49, 1.23 (each, 3 H, C(CH₃)₂(C₂H₅)) signals at 4.81 s [D₂O exchangeable]; MS (EI, 30 eV, 220 °C) m/e 337 (3.3), 336 (8.8, M⁺), 321 (70.0), 278 (12.8), 249 (10.8), 248 (14.3), 247 (100), 187 (40.7, P₂H₅), 166 (23.8, P₂H₅), 165 (10.5, P₂H₅), 81 (19.3), 43 (19.8). Anal. (C₂₄H₂₄N₄O₆S) C, H, N.

(4)-9-[8-(2a,3a-Di-O-isopropylidene-6-(hydroxymethyl)-cyclopentyl)-6-amino-8-azapurine] (18). (4)-9-[8-(2a,3a-Dihydroxy-4 β -(hydroxymethyl)cyclopentyl)-6-amino-8-azapurine] (17). 0.3 g (1.13 mmol), p -TolOH-H₂O (4.96 g, 26.1 mmol), acetone (200 mL), 2,2-dimethoxypropane (20 mL), and saturated NaHCO₃ solution (150 mL). Recrystallization from absolute ethanol provided a white solid which was identified as pure 18: total yield 0.22 g, 64%; mp 232–232 °C; $R_f = 0.66$ (chloroform-

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methanol 3.11; UV_{max} 284, 207 (0.1 N HCl); 282, 234 (sh) (H₂O); 280, 216 (0.1 N NaOH); IR (cm⁻¹) 3486, 3283, 2989, 2917 (broad, O—H, N—H, C—H); 1691, 1614, 1551 (C=N, C=C, N=N); 1276 (C(=O)H), 1325, 1218, 1070; ¹H-NMR (Me₂SO-*d*₆ + 8.45, 8.11 (each, each 1 H, C6-NH₂), 8.30 (s, 1 H, C2-H), 5.27 to 4.38 (overlapping m's, 2 H, C1'-H, C2'-H), 4.80 (t, *J* = 3.1, 1 H, C3'-H), 4.63 (m, 1 H, C3'-H), 3.50 (d, *J* = 5.1, 2 H, C4'-H), 2.46 to 2.18 (m, 3 H, C6-H₃, C4'-H), 1.48, 1.24 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signals at 8.45, 8.11, and 4.80 are D₂O exchangeable; MS (FAB, thioglycerol) 307 (M + H⁺), 303 (M - H⁻). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-(methylamino)purine (13). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (12, 0.84 g, 1.98 mmol) was combined with 40% methanamine 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 × 10 mL). The organic layers were combined and reduced to an off-white solid (0.825 g). Recrystallization from ethyl acetate-ether provided a white solid which was identified as 13: total yield 0.519 g, 85%; mp 146–147 °C; *R*_f = 0.63 (chloroform-methanol 3:1); UV_{max} 283, 210 (0.1 N HCl), 286, 208 (H₂O); 286, 218 (0.1 N NaOH); IR (cm⁻¹) 3283 (broad, O—H, N—H), 2982, 2927, 1181, 1506 (C=C, C=N), 1381 (C(CH₃)₂), 1325, 1224, 1208, 1150, 1065; ¹H-NMR (Me₂SO-*d*₆ + 8.24, 8.21 (each, each 1 H, C2 and C8H₈), 7.95 (b, 1 H, ArNH(CH₃)), 5.01 (t, *J* = 6.7, 1 H, C1'-H), 4.91 to 4.69 (overlapping m's, 2 H, C3'-H, C2'-H), 4.54 (m, 1 H, C3'-H), 2.55 (b, 1 H, C4'-H), 1.47, 1.22 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signal at 7.95 disappears and signal at 4.91 to 4.69 simplifies in D₂O; MS (EI, 50 eV, 220 °C) *m/z* 319 (5.3, M⁺), 304 (5.4), 291 (6.4), 251 (13.1), 220 (100), 205 (6.1), 204 (9.4), 176 (9.9), 150 (30.8, PH⁺), 146 (15.2, PH⁺), 120 (12.8), 91 (12.5), 43 (9.8). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-(dimethylamino)purine (14). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (12, 0.843 g, 1.98 mmol) was combined with 40% dimethylamine in water (100 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 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 (0.839 g). Recrystallization from ethyl acetate-methanol furnished a white solid which was identified as 14: total yield 0.592 g, 90%; mp 184–185 °C; *R*_f = 0.74 (chloroform-methanol 3:1); UV_{max} 286, 215 (0.1 N HCl); 278, 214, 192 (H₂O); 278, 218 (0.1 N NaOH); IR (cm⁻¹) [3232, 3089] (O—H), 2916, 2865, 1505, 1558 (C=C, C=N), 1425, 1351 (C(CH₃)₂), 1329, 1074; ¹H-NMR (Me₂SO-*d*₆ + 8.28, 8.21 (each, each 1 H, C2 and C8H₈), 5.01 (t, *J* = 6.7, 1 H, C1'-H), 4.94 to 4.69 (overlapping m's, 2 H, C3'-H, C2'-H), 4.55 (m, 1 H, C3'-H), 3.50 (m, 2 H, C4'-H), 3.45 (s, 6 H, ArNH(CH₃)₂), 2.23 (m, 3 H, C4'-H, C(CH₃)₂), 1.48, 1.23 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signal at 4.94–4.69 shrinks in size in D₂O; MS (EI, 30 eV, 150 °C) *m/z* 333 (7.5, M⁺), 318 (6.1), 276 (5.3), 245 (16.3), 244 (100), 219 (6.9), 218 (7.9), 190 (8.3), 184 (20.0, PH⁺), 134 (22.5), 43 (13.4). Anal. (C₁₄H₁₈N₆O₅) C, H, N.

Sulfamoyl Chloride. ¹ Chlorosulfamoyl isocyanate (5.6L, 67.8 mmol) was cooled to -24 °C in a dry ice-acetone/ether bath. As this cool liquid was stirred under nitrogen, concentrated hydrochloric acid (HCl, 1.8 mL, 0.15 mmol HCl and 63.0 mmol H₂O) 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 (NH₂-SO₂-Cl) and were stored in the freezer for use in future reactions.

[IR (neat, cm⁻¹) 3227, 3381, 3282, 1546, 1391, 1180, (S=O), 1066, 927. These peaks agree with the published spectrum²⁷ of 0–H, N—H, C—H); 1691, 1614, 1551 (C=N, C=C, N=N); 1276 (C(=O)H), 1325, 1218, 1070; ¹H-NMR (Me₂SO-*d*₆ + 8.45, 8.11 (each, each 1 H, C6-NH₂), 8.30 (s, 1 H, C2-H), 5.27 to 4.38 (overlapping m's, 2 H, C1'-H, C2'-H), 4.80 (t, *J* = 3.1, 1 H, C3'-H), 4.63 (m, 1 H, C3'-H), 3.50 (d, *J* = 5.1, 2 H, C4'-H), 2.46 to 2.18 (m, 3 H, C6-H₃, C4'-H), 1.48, 1.24 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signals at 8.45, 8.11, and 4.80 are D₂O exchangeable; MS (FAB, thioglycerol) 307 (M + H⁺), 303 (M - H⁻). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

General Procedure for Sulfamoylation. A suspension of compound 7, 9, 13, 15, or 18, sodium hydride (NaH), and anhydrous ethylene glycol dimethyl ether (DME) was stirred under nitrogen at room temperature + 50–60 °C for 2 to 5 h. The suspension was cooled in an ice bath, and a solution of NH₂-SO₂-Cl 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, 18, and 19–21.

8-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-(dimethylamino)purine (10). Method A (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (10, 0.586 g, 1.35 mmol), NaH (0.300 g, 12.5 mmol), DME (50 mL), NH₂-SO₂-Cl (0.650 g) in DME (60 mL) were used. 8 was isolated as a white solid: total yield 0.113 g, 27%; mp 168 °C dec; *R*_f = 0.31 (ethyl acetate-water-1-propanol 4:2:1); UV_{max} 287, 208 (0.1 N HCl); 287, 207 (H₂O); 287, 215 (0.1 N NaOH); IR (cm⁻¹) [3323, 3145] (N—H), 2953 (C—H), 1678, 1608 (C=N, C=O), 1357, 1176 (S=O), 1138, 1034, 922; ¹H-NMR (Me₂SO-*d*₆ + 8.28, 8.16 (each, each 1 H, C2 and C8 H₈), 7.47 (s, SO₂NH₂), 7.28 (s, 2 H, C6-NH₂), 5.57 (s, 2 H, C1'-H), 4.09, 3.75 (each, each 2 H, C2'-H and C3'-H); signals at 7.47 and 7.28 are D₂O exchangeable; MS (FAB, thioglycerol) 388 (M + H⁺). Anal. (C₁₄H₁₈N₆O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(dimethylamino)purine (10). Method A (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (10, 0.586 g, 1.35 mmol), NaH (0.300 g, 12.5 mmol), DME (50 mL), NH₂-SO₂-Cl (0.650 g) in DME (60 mL). 18 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} 289, 212 (0.1 N HCl); 282, 206 (H₂O); 287, 218 (0.1 N NaOH); IR (cm⁻¹) [3492, 3347, 3309, 3145] (N—H), 2988, 2940 (C—H), 1689, 1608 (C=C, C=N), 1575 (C(=O)H), 1308, 1180 (S=O), 1074; ¹H-NMR (Me₂SO-*d*₆ + 8.26, 8.13 (each, each 1 H, C2 and C8H₈), 7.52 (s, 2 H, C6-NH₂), 7.21 (s, 2 H, C6-NH₂), 5.05 (m, 1 H, C1'-H), 4.94 (m, 1 H, C2'-H), 4.68 (m, 1 H, C2'-H), 4.15 (d, *J* = 6.3, 2 H, C3'-H), 2.50 to 2.22 (m, 3 H, C4'-H, C(CH₃)₂), 1.49, 1.24 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signals at 7.52 and 7.21 are D₂O exchangeable; MS (FAB, thioglycerol) 388 (M + H⁺), 380 (M - H⁻), 419 (M + Cl⁻). Anal. (C₁₄H₁₈N₆O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(dimethylamino)purine (19). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (13, 0.491 g, 1.54 mmol), NaH (1.0–1.50 g, 8.26 mmol), DME (60 mL), and NH₂-SO₂-Cl (0.450 g) in DME (80 mL) were used. Column fractions containing 19 were combined and reduced to a white solid (478 mg). Recrystallization from methanol-ether provided 19 as a white solid: total yield 0.280 g, 47%; mp 180 °C dec; *R*_f = 0.65 (ethyl acetate-methanol 1:1); UV_{max} 283, 210 (0.1 N HCl); 289, 208 (H₂O); 287, 218 (0.1 N NaOH); IR (cm⁻¹) [3382, 3217, 3001] (N—H), 1627, 1574 (C=C, C=N), 1590 to 1352 (S=O and C(CH₃)₂), 1323, 1176 (S=O), 1082; ¹H-NMR (Me₂SO-*d*₆ + 8.26, 8.22 (each, each 1 H, C2 and C8H₈), 7.70 (b, 1 H, ArNH(CH₃)), 7.63 (s, 2 H, H₂NSO₂), 5.07 (m, 1 H, C1'-H), 4.88 (m, 1 H, C2'-H), 4.60 (m, 1 H, C2'-H), 4.15 (d, *J* = 6.5, 2 H, C3'-H), 2.97 (s, 3 H, NH(CH₃)), 2.50 to 2.23 (m, 3 H, C4'-H, C(CH₃)₂), 1.49, 1.24 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signals at 7.70 and 7.50 are D₂O exchangeable; MS (FAB, thioglycerol) 399 (M + H⁺). Anal. (C₁₄H₁₈N₆O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(dimethylamino)purine (20). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (15, 0.471 g, 1.4 mmol), NaH (1.0–2.00 g, 8.3

mmol), DME (60 mL), and NH₂-SO₂-Cl (0.400 g) in DME (80 mL) were used. 20 was identified as a white solid: total yield 0.384 g, 56%. Analytical samples were prepared by recrystallization from methanol-water (mp 215 °C dec) and from methanol (mp 185 °C dec); *R*_f = 0.79 (ethyl acetate-methanol 1:1); UV_{max} 283, 287 (sh), 227, 201 (0.1 N HCl); 285 (sh), 286, 220, 184 (H₂O); 286, 222 (0.1 N NaOH); IR (cm⁻¹) [3296, 3191, 3145] (N—H), 2988, 2909, 1572 (C=C, C=N), 1574 (C(CH₃)₂), 1308, 1178 (S=O), 1072; ¹H-NMR (Me₂SO-*d*₆ + 8.24, 8.18 (each, each 1 H, C2 and C8H₈), 7.64 (s, 2 H, H₂NSO₂), 5.07 (m, 1 H, C1'-H), 4.96 (m, 1 H, C2'-H), 4.81 (m, 1 H, C2'-H), 4.15 (d, *J* = 6.5, 2 H, C3'-H), 2.67 (s, 3 H, C6-NH₂), 2.50 to 2.18 (m, 3 H, C4'-H, C(CH₃)₂), 1.51, 1.25 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signal at 7.64 is D₂O exchangeable; MS (FAB, thioglycerol) 418 (M + H⁺), 414 (M - H⁻), 450 (M + Cl⁻). Anal. (C₁₄H₁₈N₆O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-amino-8-azapurine (21). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-amino-8-azapurine (21, 0.549 g, 1.13 mmol), NaH (1.0–1.50 g, 8.26 mmol), DME (60 mL), and NH₂-SO₂-Cl (0.300 g) in DME (80 mL) were used. Impure product was collected as a white solid (0.864 g). Recrystallization of this solid from methanol furnished pure 21: total yield 0.574 g, 63%; mp 225 °C; *R*_f = 0.76 (ethyl acetate-methanol 1:1); UV_{max} 284, 207 (0.1 N HCl); 279, 203 (H₂O); 279, 216 (0.1 N NaOH); IR (cm⁻¹) [3357, 3286, 3101] (N—H), 1677, 1605, 1572 (C=C, C=N, N=N), 1574 (C(CH₃)₂), 1322, 1184 (S=O), 1077; ¹H-NMR (Me₂SO-*d*₆ + 8.44, 8.11 (each, each 1 H, C2 and C8H₈), 8.31 (s, 1 H, C2'-H), 5.50 (s, 2 H, H₂NSO₂), 5.19 to 4.07 (overlapping m's, 2 H, C1'-H, C2'-H), 4.67 (m, 1 H, C2'-H), 4.15 (d, *J* = 5.7, 2 H, C3'-H), 2.56 (m, 3 H, C4'-H), 1.58 (each, each 3 H, C(CH₃)₂, C(CH₃)₂); signals at 8.44, 8.11, and 7.52 are D₂O exchangeable; MS (FAB, thioglycerol) 386 (M + H⁺), 384 (M - H⁻). Anal. (C₁₄H₁₈N₆O₅) C, H, N.

Method B Used To Make 10. (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (10, 0.599 g, 1.31 mmol) was combined with bis(trimethylsilyl) oxide (2.3 g, 3.8 mmol) and anhydrous benzene (40 mL), and the mixture was refluxed under nitrogen for 2 h. The mixture, which becomes a solution while refluxing, was then cooled in an ice bath. NH₂-SO₂-Cl (0.450 g) dissolved in anhydrous dioxane (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 NH₂-SO₂-Cl (0.600 g) in anhydrous dioxane (31 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 (6 g). The silica sample was placed on a silica column (60 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 285 °C.

General Procedure for Removal of the Acetonide. Compound 10, 18, 20, or 21 was dissolved in 90% aqueous trifluoroacetic acid (CF₃CO₂H/H₂O) 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-NH₃, 1:1). The solvent was again removed, and the remaining syrup was crystallized from water to provide 2, 3, 5, and 6.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(dimethylamino)purine (2). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-thioguanosine (10, 0.517 g, 1.38 mmol), CF₃CO₂H-H₂O (30 mL) and MeOH-NH₃ (20 mL) were used. 2 was isolated as an off-white solid: total yield 0.302 g, 65%; mp 157–158 °C; *R*_f = 0.19 (ethyl acetate-water-propanol 4:2:1); UV_{max} 289, 211 (0.1 N HCl); 281, 206, 193 (H₂O); 281, 216 (0.1 N NaOH); IR (cm⁻¹) [3413, 3139 (broad)] (O—H, C—H), 1665, 1611, 1577 (C=N, C=C), 1354, 1188 (S=O); ¹H-NMR (Me₂SO-*d*₆ + 8.18, 8.11 (each, each 1 H, C2 and C8H₈), 7.49 (s, 2 H, H₂NSO₂), 7.18 (s, 2 H, C6-NH₂), 5.07 (d, *J* = 6.4, 1 H, C2'-H), 4.89 (d, *J* = 4.4, 1 H, C3'-H), 4.69 (m, 1 H, C3'-H), 4.38

(m, 1 H, C3'-H), 4.16, 4.07 (each, each 1 H, C3'-H, C4'-H), 3.87 (m, 1 H, C3'-H), 2.31 (m, 2 H), 1.78 (m, 1 H) (C8-H₈ and C4'-H); signals at 7.49, 7.18, 5.07, and 4.89 are D₂O exchangeable; MS (FAB, thioglycerol) 348 (M + H⁺), 343 (M - H⁻), 379 (M + Cl⁻). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(methylamino)purine (3). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-(methylamino)purine (3, 0.491 g, 1.54 mmol), NaH (1.0–1.50 g, 8.26 mmol), DME (60 mL), and NH₂-SO₂-Cl (0.450 g) in DME (80 mL) were used. Impure product was collected as a white solid: total yield 0.184 g, 72%; mp 179 °C dec; *R*_f = 0.35 (concentrated ammonia-propanol 1:4); *R*_f = 0.35 (concentrated ammonia-propanol 1:4); UV_{max} 284, 211 (0.1 N HCl); 287, 209 (H₂O); 287, 218 (0.1 N NaOH); IR (cm⁻¹) [3446, 3273, 3182] (broad, N—H, C—H), 1628 (C=C, C=N), 1382, 1186 (S=O); ¹H-NMR (Me₂SO-*d*₆ + 8.19, 8.15 (each, each 1 H, C2 and C8H₈), 7.82 (s, 1 H, ArNH(CH₃)), 7.47 (s, 2 H, H₂NSO₂), 5.35 (d, *J* = 6.4, 1 H, C2'-H), 4.87 (d, *J* = 4.4, 1 H, C3'-H), 4.70 (m, 1 H, C3'-H), 4.37 (m, 1 H, C2'-H), 4.15, 4.07 (each, each 1 H, C3'-H, C4'-H), 3.87 (m, 1 H, C3'-H), 2.56 (m, 3 H, C(CH₃)₂, C(CH₃)₂), 2.30 (m, 2 H), 1.80 (m, 1 H) (C6-H₃ and C4'-H); signals at 7.82, 7.47, 6.05, and 4.87 are D₂O exchangeable; MS (FAB, thioglycerol) 359 (M + H⁺), 357 (M - H⁻). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(methylthio)purine (4). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-methylthiopurine (4, 0.373 g, 0.96 mmol), CF₃CO₂H-H₂O (15 mL), and MeOH/NH₃ (10 mL) were used. 4 was isolated as an off-white solid: total yield 0.307 g, 61%; mp 147–150 °C dec; *R*_f = 0.38 (concentrated ammonia-propanol 1:4); *R*_f = 0.39 (ethyl acetate-water-propanol 4:2:1); UV_{max} 295, 223, 202 (0.1 N HCl); 291, 286, 214, 183 (H₂O); 292, 286, 222 (0.1 N NaOH); IR (cm⁻¹) [3296 (broad, O—H), 1581, 1561] (C=C, C=N), 1359, 1184 (S=O), 1074; ¹H-NMR (Me₂SO-*d*₆ + 8.73, 8.54 (each, each 1 H, C2 and C8H₈), 7.93 (s, 2 H, H₂NSO₂), 5.13 (d, *J* = 6.3, 1 H, C2'-H), 4.96 (d, *J* = 4.3, 1 H, C3'-H), 4.82 (m, 1 H, C1'-H), 4.63 (m, 1 H, C2'-H), 4.12 (m, 2 H, C3'-H), 3.80 (m, 1 H, C3'-H), 2.56 (s, 3 H, C6-NH₃), 2.33 (m, 2 H), 1.82 (m, 1 H) (C6-H₃ and C4'-H); signals at 7.93, 8.15, and 4.96 are D₂O exchangeable; MS (FAB, thioglycerol) 376 (M + H⁺), 374 (M - H⁻), 410 (M + Cl⁻). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(dimethylamino)purine (6). (4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(hydroxymethyl)-cyclopentyl)]-6-amino-8-azapurine (21, 0.23 g, 0.46 mmol), CF₃CO₂H-H₂O (15 mL), and MeOH-NH₃ (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} 283, 207 (0.1 N HCl); 279, 204 (H₂O); 287, 216 (0.1 N NaOH); IR (cm⁻¹) [3182 (broad, O—H, N—H), 1700, 1617, 1573] (C=C, C=N, N=N), 1580, 1574, 1504, 1382 (S=O), 1382 (C(CH₃)₂), 1324, 1182 (S=O); ¹H-NMR (Me₂SO-*d*₆ + 8.31, 8.06 (each, each 1 H, C2 and C8H₈), 8.28 (s, 1 H, C2'-H), 7.49 (s, 2 H, H₂NSO₂), 5.16 (d, *J* = 6.1, 1 H, C2'-H), 4.96 (m, 1 H, C1'-H), 4.79 (m, 1 H, C2'-H), 4.36 (m, 1 H, C3'-H), 4.18, 4.11 (each, each 1 H, C3'-H, C4'-H), 3.89 (m, 1 H, C3'-H), 2.56 (m, 3 H, C(CH₃)₂, C(CH₃)₂), 2.30 (m, 2 H), 1.80 (m, 1 H) (C6-H₃ and C4'-H); signals at 7.49, 8.15, and 4.96 are D₂O exchangeable; MS (FAB, thioglycerol) 346 (M + H⁺). Anal. (C₁₂H₁₄N₄O₅) C, H, N.

(4)-*β*-[2-(2,3,6-Di-O-isopropylidene-4β-(sulfamoyl)-cyclopentyl)]-6-(dimethylamino)purine (14). (4)-*β*-[2-(2,3,6-D

each 1 H, C2 and C8/H), 7.52 (s, 2 H, H_2 NSO₂), 1.48, 1.23 (each s, each 3 H, CH₃), (CH₃)₂ 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 = 0.35 (concentrated ammonia prepapal 14); UV_{max} 269, 213 (0.1 N HCl); 277, 213 (H₂O); 276, 119 (0.1 N NaOH); IR (cm⁻¹) (3177, 1144) (broad, O–H); (1668, 1565) (C=O, C=N), 1423, 1343, 1182 (S=O); ¹H NMR (Me₂SO-*d*₆) δ 8.19 (s, 2 H, C2 and C8/H), 7.49 (s, 2 H, H_2 NSO₂), 5.05 (d, J = 6.5, 1 H, C2/H), 4.89 (d, J = 4.5, 1 H, C2/H), 4.79 (m, 1 H, C2/H), 4.36 (m, 1 H, C2/H), 4.16, 4.07 (each m, each 1 H, C2/H), 3.87 (m, 1 H, C2/H), 3.45 (s, 6 H, ArN(CH₃)₂), 2.32 (m, 2 H), 1.74 (m, 1 H) (C2/H and C8/H); signals at 7.48, 5.05, and 4.89 are D₂O exchangeable; MS (FAB, thioglycerol) 373 (M⁺ + H⁺). Anal. (C₁₂H₁₂N₄O₅S) C, H, N.

Biology. The Fischer 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 [¹⁴C]-uridine (68.84 Ci/mmol), [¹⁴C]-leucine (303 mCi/mmol), and [¹⁴C]-lysine (306 mCi/mmol) were obtained from New England Nuclear, Inc. The [¹⁴C]-thymidine (61.4 mCi/mmol) was purchased from Amersham, and the [¹⁴C]-L-phenylalanine (465 mCi/mmol) was purchased from ICN. Centrifugation was done on a Sorvall RC29B instrument. Liquid scintillation was purchased using a Beckman LS 6800 counter. HA type filters were purchased from Millipore Corp. Eosline 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* B10 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 *in vitro* antitumor agents by using the protocol of the National Cancer Institute.¹⁹ The methodology used was described in ref. 31. P388 Mouse Leukemia cells grown in Fibers 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 purinomycin was used as a positive control. The approximate concentration of each test compound which inhibits cell growth by 50% (IC₅₀) is reported in Table I.

Assay for DNA, RNA, and Protein Biosyntheses. A modified Fibers media was prepared which contained only half the recommended concentrations of L-phenylalanine (Pha, 30 mg/L used), L-leucine (Leu, 15 mg/L used), and L-lysine (Lys, 25 mg/L used). For the protein assay, 10⁵ each of [¹⁴C]-Pha, [¹⁴C]-Leu, and [¹⁴C]-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 five 2-mL portions. To one of these portions, the compound to be tested (2 or 8) 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 10^5 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 270g 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 filter (0.45 µm). The filter was washed with 2 × 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 acid 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 [¹⁴C]amino acids were replaced with [¹⁴C]-2-uridine for the RNA assay and [¹⁴C]-thymidine for the DNA assay. The results of the studies are depicted in Figures 1, 2, and 3.

Polydeoxyriboside-1-uridylic Acid-Directed Synthesis of Polypeptides. The *E. coli* protein biosynthesis assays were essentially as described previously.^{42,43} Compounds 2 and 6 and purinomycin 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^{42,43} devoid of the messenger RNA and also containing [¹⁴C]-Pha. Various concentrations of the test compounds were prepared. Protein biosynthesis was initiated by adding polydeoxyriboside-1-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 (2 × 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 [¹⁴C]-Pha 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 purinomycin 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* B10 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). 5-fold serial dilutions of the agents in 1.5 mL of water were prepared in sterile tubes equipped with sterile cotton plugs. An inoculum of an overnight culture of bacterial was suspended in 2X nutrient broth media, and 1.5 mL of this suspension was added to each tube in the series. The 5-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 Reticuloendothelial 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

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. [¹⁴C]-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 cyclohexanamide 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 munit/ml) 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 standard assay procedure, 1 mL of citric acid buffer (0.01 M, pH 6.5) and rabbit muscle adenylic acid deaminase (50 munit/ml) 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.

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