Small-Molecule Immunostimulants. Synthesis and Activity of 7,8-Disubstituted Guanosines and Structurally Related Compounds

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A series of 7,8-disubstituted guanosine derivatives was designed and prepared as potential B-cell-selective activators of the humoral immune response. These compounds were evaluated for their ability to act as B-cell mitogens and to augment the antibody response of B cells to sheep red blood cell (SRBC) challenge (adjuvanticity). In addition, they were tested for their ability to stimulate the natural killer (NK) cell response in murine in vitro cell assays. Certain of the compounds demonstrated in vivo activity when administered either intravenously, subcutaneously, or orally. Analogues with a medium-length alkyl chain (2–4 carbons, **5–7**) on the 7-position of 7-alkyl-8-oxoguanosines were found to be particularly potent. Compounds bearing hydroxyalkyl, aminoalkyl, or substituted aminoalkyl substituents on this 7-position were weakly active. However, benzyl groups, including those substituted with heteroatoms (e.g., *p*-nitrobenzyl, **14**), were active. Oxo, thioxo, and seleno groups on C-8 of the guanosine ring all imparted strong activity, whereas other larger substituents did not (e.g., N=CN). Stereochemical inversion of the 2′-hydroxyl on the ribose ring in this series, giving arabinose analogue **70**, lessened activity. However, removal of the 2′-hydroxyl, either with (**64**) or without (**73**) removal of the 3′-hydroxyl, resulted in excellent activity and improved solubility; **64** also displayed good oral in vivo activity as well. A series of ketals involving the 2′,3′-hydroxyls were prepared; certain of the nonpolar ketals (e.g., **48**) were remarkably active, pointing to an ancillary hydrophobic binding region that can augment activity. 5′-Phosphate derivative **57** was fairly active, and acyclovir analogue **90** displayed good NK-selective activity: other N-9 sugar mimetics were also active (**97-104**), although this activity did not carry over into the human B-cell assay. A total of 80 compounds were prepared and evaluated for their immunostimulating activity. Within this group, compounds could be divided into those that were active in all three assays, those that displayed some measure of selectivity for the adjuvanticity assay, and those that preferentially activated NK responses. Because of its overall biological profile and ease of synthesis, 7-allyl-8-oxoguanosine (**6**; loxoribine, RWJ-21757) was chosen for further development. It is among the most potent compounds evaluated in the three biological assays.

The search for agents that restore or enhance the ability of the human immune system to ward off infection or other invasive challenges is a key aspect of therapeutic research. The AIDS epidemic and the need for adjuvant therapy to boost the immune system of the elderly and cancer patients has brought this area of immunopotentiation into sharp focus.[1,2] Many different types of compounds have been demonstrated to possess immunostimulatory properties. These include glycoproteins such as interferon,[3] peptides such as tuftsin,[3,4] polynucleotides,[3] and small heterocycles such as levamisole.[3,5] From our standpoint, nucleoside-based small-molecule immunostimulants that affect the proliferation and potentiation of the cytokine-producing humoral cells offer considerable promise.[6] The activity of 8-bromoguanosine (**1**), first prepared by Holmes and Robbins in 1965,[7] has led to the preparation and evaluation of other 8-substituted guanosines. For example, the synthesis and testing of 7-methyl-8-oxoguanosine (**2**)[8] and 7-thia-8-oxoguanosine (**3**)[9] have already been reported. Compounds **1-3** are intracellular mitogens for B lymphocytes and stimulate the proliferation of B cells in the presence of antigenic challenge.[6] In addition, **3** activates murine NK cells in an assay measuring the destruction of a Yac tumor cell line.[9] Bropirimine (**4**) is structurally related to nucleosides **1-3** in that it bears a 2-amino-6-pyrimidone ring system, although it lacks a ribose ring. It is a small-molecule interferon inducer discovered by Wierenga and co-workers at Upjohn and has been evaluated in clinical trials for various indications in the antiviral and anticancer areas.[10]

At the point our work was initiated, one of the key messengers mediating B cell activation was thought to be guanosine 3′,5′-monophosphate (cGMP).[11] Studies at the Scripps Research Institute by Goodman and Weigle indicated that 8-bromo-cGMP induces a 3–5-fold

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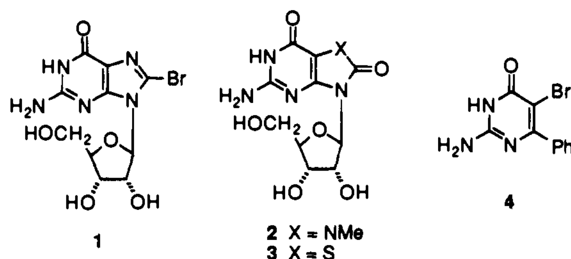


stimulation of B-cell proliferation.[12] Further research showed that 8-bromoguanosine (1) was 50–100 times more potent in the stimulation of B lymphocytes than 8-bromo-cGMP.[13] These C-8 substituted guanosines were transported across the cell membrane prior to mediating their proliferative action.[14] However, they were not substrates for guanylate cyclase and do not enhance the activity of this enzyme.[15]

As part of an ongoing Johnson & Johnson–Scripps research collaboration, we have pursued the area of immunopotentiation toward a therapeutic endpoint.[16] This paper describes our structure–activity relationship (SAR) studies starting with guanosine 2 as a lead compound and systematically modifying the molecule. We have varied substituents on the purine ring and the ribose portion of the molecule. Our goal has been to obtain a B-cell-selective immunostimulant which could have use as a vaccine adjuvant, in states of immunodeficiency (e.g., AIDS, common variable immunodeficiency), and possibly in cancer. Also, we have sought to obtain solubility properties amenable to efficient drug delivery, an important concern in the area of guanosine-based drugs.

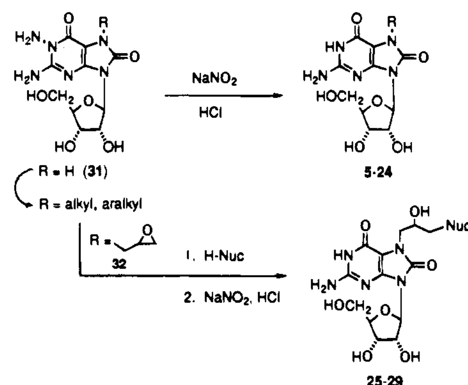
Chemical Synthesis

As the N-7 methyl group of 2 imparted a large biological advantage when compared with the parent structure 8-oxoguanosine,[8a] manipulation of the N-7 substituent was an obvious starting point for our research. We have also prepared a variety of other structurally-modified guanosine derivatives, which are listed in Tables 1–4 and in the schemes.

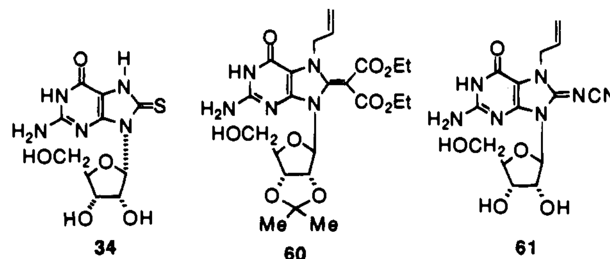
Common intermediate 31[8b] was synthesized and alkylated with a variety of electrophiles and deprotected as required. The final targets were generally purified by reversed-phase semipreparative HPLC (see Scheme 1). We synthesized 5-24 by this method. A variety of different alkyl and benzyl electrophiles were employed in the construction of the target compounds. The use of racemic epibromohydrin as an electrophile resulted in epoxide 32, which was somewhat unstable to prolonged storage, but could be directly reacted with nucleophiles to prepare compounds 26-29 as equal mixtures of diastereomers after removal of the N-1 protecting group. Deprotection of the amino group at N-1 was conducted with sodium nitrite and HCl. An attempt to purify (reversed-phase HPLC, water) a crude batch of 32, which had been subjected to the deprotection reaction at N-1, resulted in opening of the epoxide to give 25. In addition, amine 30 was synthesized from azide 29 by catalytic reduction (H_2, Pd/C). Compounds prepared by these chemical methods are listed in Table 1.

When it became apparent that a medium alkyl chain on N-7 (2–4 carbons) was required for optimal biological activity,[16a] we sought an improved synthetic route for

Scheme 1



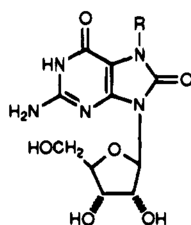
a potential clinical candidate. 8-(Allyloxy)guanosine (33) was obtained by displacement of 8-bromoguanosine (1) with the anion of allyl alcohol (see Scheme 2, 70%). The optimum conditions for this reaction were the use of a mixture of DMSO and allyl alcohol as cosolvents; attempts to limit the use of allyl alcohol to ca. 5 molar equiv led to considerable chemical decomposition. 3,3-Sigmatropic rearrangement of 33 in refluxing water/methanol (1:1) afforded 7-allyl-8-oxoguanosine (6).[17a] This route was also applied to the preparation of the 8-thio and 8-seleno analogues 37 and 38, respectively. Compound 1 was converted to 8-thioguanosine (35) by treatment with thiourea. For the sake of comparison, an analogue of 37 was prepared (34) in which the ribose ring had the α sugar stereochemistry. This material was synthesized from α-guanosine, prepared by a new method that we developed.[16b] Conversion of α-guanosine to the 8-bromo derivative was followed by treatment with thiourea as for 35 to give 34. Compound 35 was allylated with allyl bromide to yield 36. The 3,3-sigmatropic rearrangement of 36 afforded 37 (130 °C, DMF, 55%) under conditions that were more vigorous than those required for 33. The analogous allylation reaction on 34 was successful; however, the conditions required for the rearrangement to the 7-allyl derivative for the α derivative were too severe, causing decomposition of the molecule. The seleno analog 38 was prepared in an analogous manner as 37.



Several peracylated derivatives of loxoribine and 37 were also prepared by reaction of these compounds with acyl chlorides or anhydrides to give compounds 39-42 (see Scheme 3 and Table 2). The N-2 amino group of 37 is slower to react with acetyl chloride than the hydroxyls on the ribose ring, so triacetate 39 was isolated in the same reaction in which 40 was prepared. Structures 39-42, as expected, were more soluble in organic solvents. They could be prodrugs for the release of the parent compounds by the in vivo action of esterase enzymes.

In order to modify the 5′-hydroxyl, we blocked the 2′- and 3′-hydroxyls with the isopropylidene ketal, as in 43

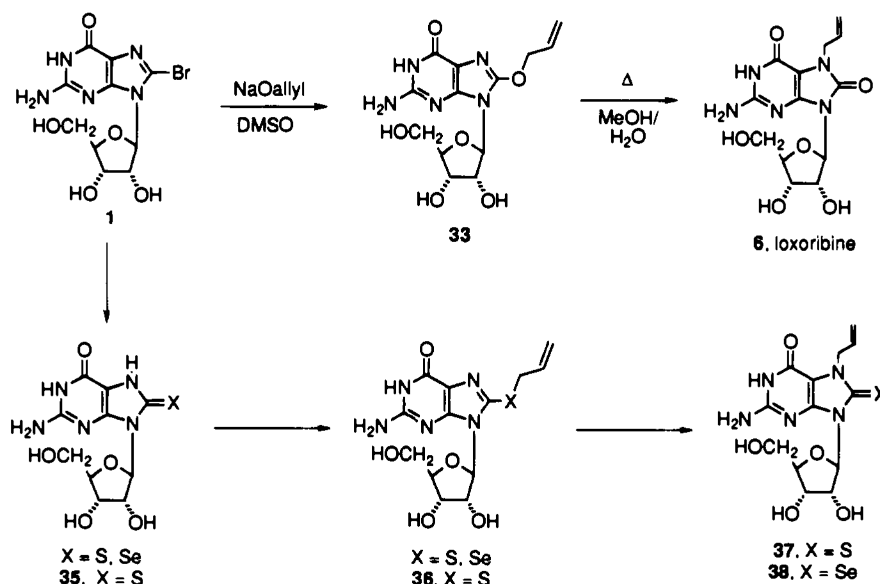
Table 1. 7,8-Disubstituted Guanosine Derivatives



compd	*R*	mp (°C)	formula[a]
5	Et	>230	$C_{12}H_{17}N_5O_6 \cdot 0.5H_2O$
6	allyl	>230	$C_{13}H_{17}N_5O_6$
7	Bu	>230	$C_{14}H_{21}N_5O_6$
8	C_6H_{13}	196-199	$C_{16}H_{25}N_5O_6$
9	$C_{10}H_{21}$	174-176	$C_{20}H_{33}N_5O_6 \cdot 0.5H_2O$
10	(*E*)-cinnamyl	>230	$C_{19}H_{21}N_5O_6$
11	$PhCH_2$	175 (softens), >230	$C_{17}H_{19}N_5O_6$
12	4-(MeO)$PhCH_2$	237-238	$C_{18}H_{21}N_5O_7 \cdot 0.5H_2O$[b]
13	4-Cl$PhCH_2$	>230	$C_{17}H_{18}ClN_5O_6 \cdot H_2O$
14	4-(NO_2)$PhCH_2$	265-267	$C_{17}H_{18}N_6O_8 \cdot 0.5H_2O$
15	4-($PhCH_2O$)$PhCH_2$	136-138	$C_{24}H_{25}N_5O_7 \cdot H_2O$
16	3,4-Cl_2PhCH_2	>230	$C_{17}H_{17}Cl_2N_5O_6$
17	3-CN$PhCH_2$	>230	$C_{18}H_{18}N_6O_6 \cdot 0.5H_2O$
18	4-CN$PhCH_2$	>230	$C_{18}H_{18}N_6O_6 \cdot 0.25H_2O$
19	4-ClPhC(O)CH_2	151-153	$C_{18}H_{18}ClN_5O_7$
20	EtO_2CCH_2	167-172	$C_{19}H_{21}N_5O_6$
21	$H_2NC(O)CH_2$	245-247	$C_{12}H_{16}N_6O_7 \cdot 0.5H_2O$[c]
22	$Me_2N(CH_2)_3$	180 dec	$C_{15}H_{24}N_6O_6 \cdot HCl \cdot 2H_2O$
23	(piperidino)N-$(CH_2)_2$	157 dec	$C_{17}H_{26}N_6O_6 \cdot HCl \cdot H_2O$
24	$Cl(CH_2)_2$	192 dec	$C_{12}H_{16}ClN_5O_6 \cdot 1.5H_2O$[d]
25	$HOCH_2CH(OH)CH_2$	195-196	$C_{13}H_{19}N_5O_8 \cdot H_2O$
26	$PhSCH_2CH(OH)CH_2$	180-182	$C_{19}H_{23}N_5O_7S \cdot 0.5H_2O$
27	3,4-$(MeO)_2Ph(CH_2)_2NHCH_2CH(OH)CH_2$	163-170	$C_{23}H_{32}N_6O_9 \cdot HCl \cdot H_2O$
28	*p*-FPh-N(piperazine)N-$CH_2CH(OH)CH_2$	178-181	$C_{23}H_{30}FN_7O_7 \cdot 0.5H_2O$
29	$N_3CH_2CH(OH)CH_2$	138-141	$C_{13}H_{18}N_8O_7 \cdot H_2O$[e]
30	$H_2NCH_2CH(OH)CH_2$	150 dec	$C_{13}H_{20}N_6O_7 \cdot HCO_2H \cdot 0.75H_2O$

[a] Where the formula is given as symbols of elements in Tables 1-4, the elemental analysis for those elements were within ±0.4% of the calculated values. [b] C: calcd, 54.66; found, 54.17. [c] C: calcd, 47.85; found, 47.32. [d] C: calcd, 37.07; found, 36.65. [e] C: calcd, 37.50; found, 38.06.

Scheme 2



(see Scheme 3 and Table 3). However, this compound and related structures displayed enough interesting activity on their own to warrant the synthesis of a number of structural variants (e.g., **44-52**). These ketals are probably not prodrugs by virtue of ketal cleavage, as this functionality is stable to dilute acid (pH 2.0, 2 h, 24 °C), and we are not aware of an enzyme that would effect cleavage (e.g., convert **43** to **2**). In addition, the known bis-ketal sugar anticonvulsant topiramate exerts its pharmacological effect with its isopropylidene groups

Scheme 3

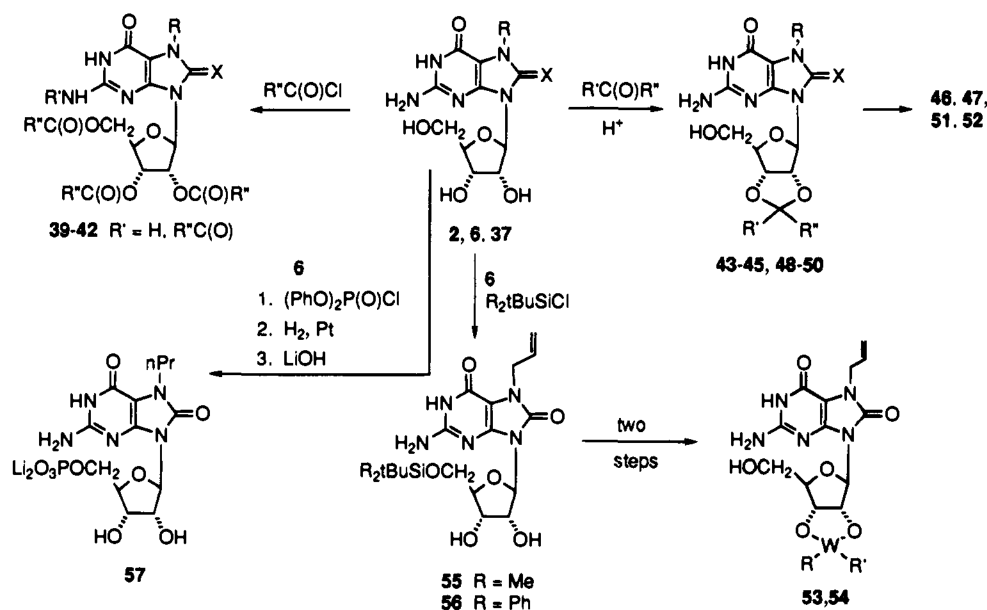
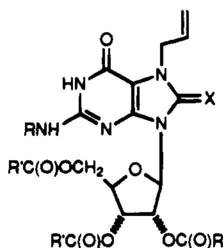


Table 2. Acylated 7,8-Disubstituted Guanosine Derivatives



compd	R	R′	X	mp (°C)	formula
39	H	Me	S	100–102	$C_{19}H_{23}N_5O_8S$
40	Ac	Me	S	90–92	$C_{21}H_{25}N_5O_9S$
41	PrC(O)	Pr	O	81–83	$C_{29}H_{41}N_5O_{10}$
42	$MeOCH_2C(O)$	$MeOCH_2$	O	–	$C_{25}H_{33}N_5O_{14}$

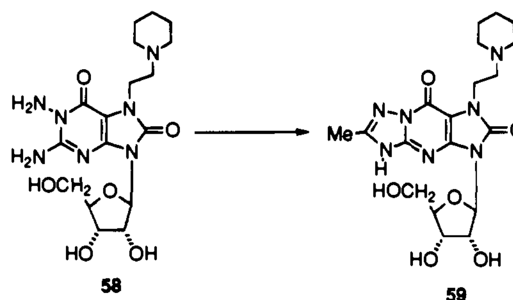
intact.[18] Compounds **43-45** were prepared directly by reaction of the parent compounds with acetone by using concentrated H_2SO_4 or zinc chloride as catalyst. The 5′-hydroxyl of **44** was further modified by acetylation and benzoylation to give **46** and **47**, respectively, which were evaluated.

It was considerably more difficult to prepare ketals larger than the isopropylidene group on the 2′- and 3′-hydroxyls, as a variety of conditions proved unsuccessful, partly because loxoribine and derivatives do not dissolve in solvents normally employed for this reaction (e.g., benzene). The best conditions were those of Seela and Waldek, who reacted guanosine with ethyl levulinate in the presence of HCl/dioxane and triethyl orthoformate.[19] With the use of this method, ketals **48-50** were synthesized from **6** and the appropriate ketones. Compounds **49** and **50** existed as an 88:12 mixture of diastereomers, with the *endo*-methyl diastereomer predominating. Compounds **51** and **52** were synthesized from **49** by saponification and amidolysis, respectively.

Benzylidine acetal **53** was also synthesized from **6**; in this case 5′-silyl protected alcohol **55** was employed, followed by deprotection of the silyl group with tetrabutylammonium fluoride; only a single diastereomer (unassigned stereochemistry) was observed. Compounds **55** and **56** were useful intermediates for the selective functionalization of the 2′,3′-hydroxyls of **6**. For example, carbonate **54** was prepared by treatment of **56** with carbonyldiimidazole, followed by removal of the 5′-silyl group with fluoride.

Many nucleoside drugs exert their pharmacological effect after being converted in vivo into their corresponding 5′-phosphate. In the case of the guanosine immunostimulants, 5′-phosphorylation probably does not play a major role.[20] We prepared 5′-phosphate **57** in order to investigate its biological properties. Compound **6** was converted into **57** by treatment with

Scheme 4



Scheme 5

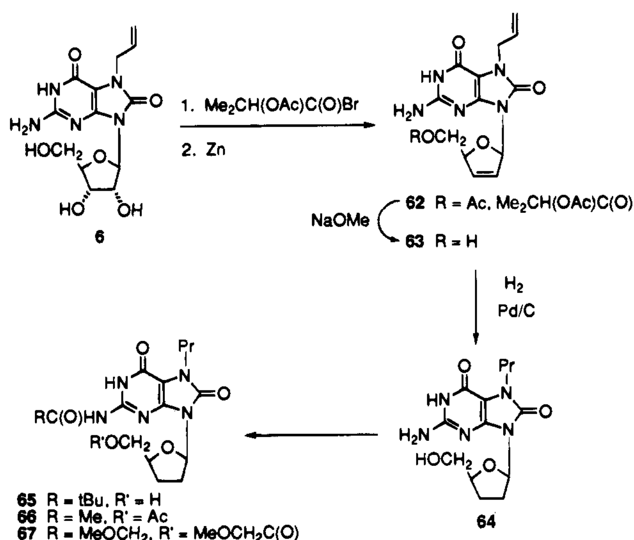
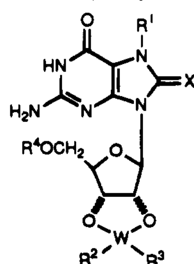


Table 3. 7,8-Guanosine Analogs Which Are Derivatized on the 2′,3′-Hydroxyls



compd	R¹	R²	R³	R⁴	W	X	mp (°C)	formula
43	Me	Me	Me	H	C	O	193 dec	$C_{14}H_{19}N_5O_6{\cdot}H_2O$
44	allyl	Me	Me	H	C	S	>230	$C_{16}H_{21}N_5O_5S{\cdot}0.5H_2O$[a]
45	(*E*)-cinnamyl	Me	Me	H	C	O	>230	$C_{22}H_{25}N_5O_6{\cdot}0.5H$
46	allyl	Me	Me	Ac	C	S	179 dec	$C_{18}H_{23}N_5O_6S$[b]
47	allyl	Me	Me	Bz	C	S	214 dec	$C_{23}H_{25}N_5O_6S{\cdot}0.25H_2O$
48	allyl	*n*-Pr	*n*-Pr	H	C	O	186–189	$C_{20}H_{29}N_5O_6$[c]
49	allyl	Me	$EtOC(O)(CH_2)_2$	H	C	O	176–179	$C_{20}H_{27}N_5O_8{\cdot}0.3H_2O$[d]
50	allyl	Me	$BnOC(O)(CH_2)_2$	H	C	O	140–142	$C_{25}H_{29}N_5O_8{\cdot}0.3H_2O$[e]
51	allyl	Me	$HO_2C(CH_2)_2$	H	C	O	239–241	$C_{18}H_{23}N_5O_8{\cdot}0.3H_2O$[f]
52	allyl	Me	$MeNHC(O)(CH_2)_2$	H	C	O	142.5–147.5	$C_{19}H_{26}N_6O_7{\cdot}0.75H_2O$
53	allyl	4-FPh	H	H	C	O	311–314	$C_{20}H_{20}FN_5O_6$[g]
54	allyl	=O		H	C	O	323–325	$C_{14}H_{15}N_5O_7{\cdot}0.2H_2O$

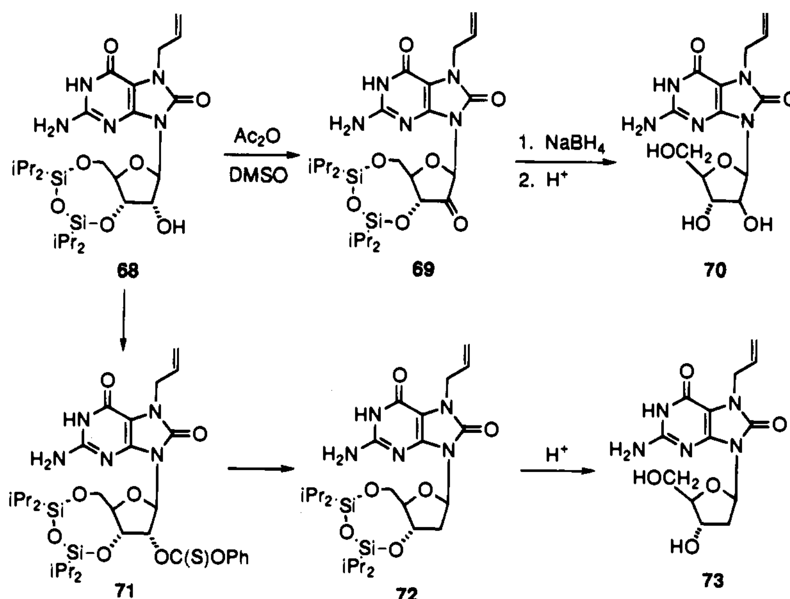
[a] C: calcd, 47.51; found, 47.08. [b] N: calcd, 16.01; found, 15.50. [c] Recrystallization solvent was EtOAc/pentane. [d] Recrystallization solvent was ether. [e] Recrystallization solvent was $CHCl_3$/hexane. [f] Recrystallization solvent was water. N: calcd, 15.79; found, 15.36. [g] Recrystallization solvent was DMSO/H_2O.

diphenyl phosphorochloridate, followed by hydrogenolytic cleavage[21] (Pt, H_2) of the phenyl phosphoryl esters and isolation as the dilithium salt.

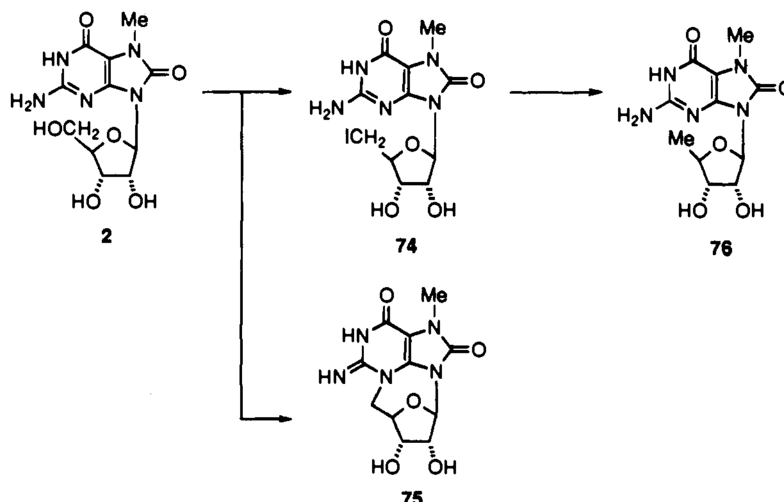
We speculated that the 2-amino-6-pyrimidinone portion of the purine ring of loxoribine and derivatives was essential for activity. Nevertheless, we did prepare one tricyclic analogue (**59**, see Scheme 4). Compound **58**, an intermediate in the preparation of **23**, was condensed with acetic anhydride followed by hydrolysis to give **59**. As **1** was also considered a lead structure, we also prepared **60** and **61** which possess other electron-withdrawing groups on the C-8 position.[22]

Dideoxy nucleoside derivatives are an important class of medicinal agents, chiefly because of their pronounced antiviral activity.[23] We explored this type of structural modification in our series as well. The activity of bropirimine (**4**),[10] which lacks the ribose ring, led us to believe that it might be possible to drastically modify the sugar portion of the loxoribine structure. Treatment of loxoribine with 1-acetoxyisobutyryl chloride[24] gave the expected mixture of vicinal bromoacetates, which were directly reduced with zinc powder to give **62** (see Scheme 5). Saponification of the 5′ ester gave unsaturated dideoxy analog **63**. Compound **63** was stable to normal laboratory manipulations, but did decompose slowly (ca. 2% in 15 h) at 70 °C. Reduction of the two olefinic double bonds of **63** was cleanly effected by hydrogenation in the presence of catalytic palladium to give **64**. We were encouraged to find that **64** displayed biological activity (see below), especially in light of improved solubility properties relative to **6**. Compound **64** was found to have >5% solubility in water at 23 °C, was freely soluble in halogenated solvents (e.g., $CHCl_3$), and was sparingly soluble in ethyl acetate. Several

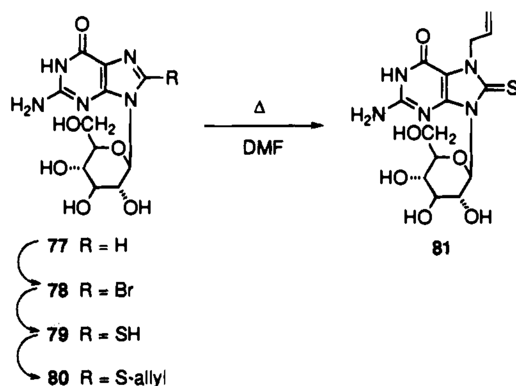
Scheme 6



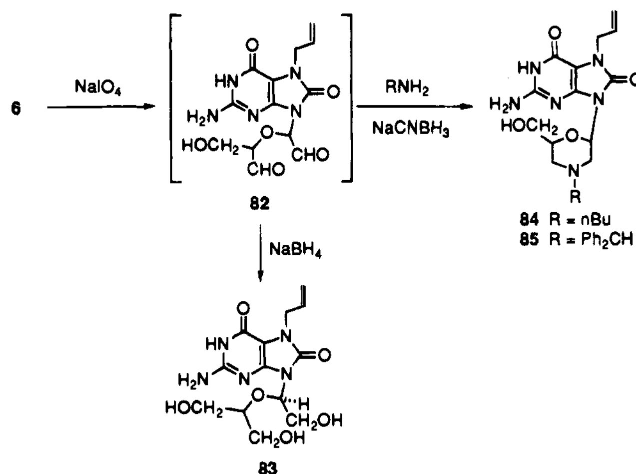
Scheme 7



Scheme 8



Scheme 9



ester and amide derivatives of **64** were prepared and evaluated (viz. **65-67**) by reaction of **63** with the appropriate acyl halides.

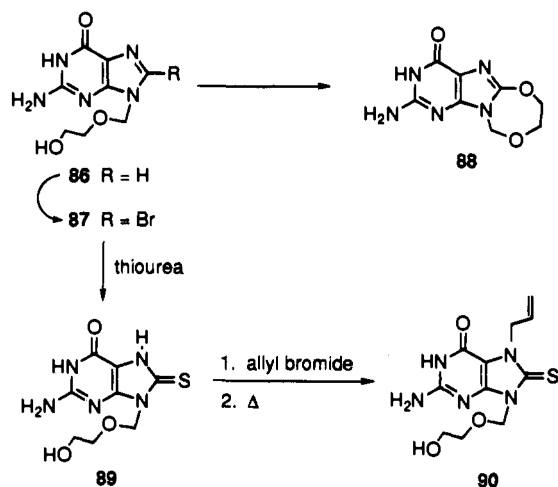
The 2'-deoxy and arabino analogues were also studied; they were prepared by the sequence of reactions shown in Scheme 6. The tetraisopropyldisiloxane (TIPS) protecting group[25] was incorporated onto loxoribine to give **68**. Oxidation of **68** using DMSO/Ac_2O resulted in a ca. 7:3 mixture of ketone **69** and the 2'-[(methylthio)methyl] ether side product.[26] As this mixture could not be readily separated, it was directly reduced with $NaBH_4$ to give a 92:8 mixture of the arabino:ribo stereoisomers, which could be readily separated from the methylthiomethyl side-product. The TIPS group was then removed under mildly acidic conditions to give a 92:8 mixture of unprotected nucleoside diastereomers (**70**: **6**), enriched in arabinose **70**. Alternatively, thiocarbonate **71** was reduced to give **72**, and removal of the TIPS group produced 2'-deoxy derivative **73**.[25]

Compound **2** was reacted with triphenylphosphine and iodine[27] in an attempt to prepare 5'-iodo derivative **74** (see Scheme 7). This latter compound was obtained (22% yield); in addition, a considerable amount of anhydro derivative **75** (41%) was isolated. Analog **75** was also tested for biological activity, as it explores the necessity of a 5'-hydroxyl group in **2**, as well as constraining the ribose ring to a position proximal to the pyrimidine portion of the purine. Iodide **74** was reduced with tributyltin hydride to give 5'-deoxy congener **76**.

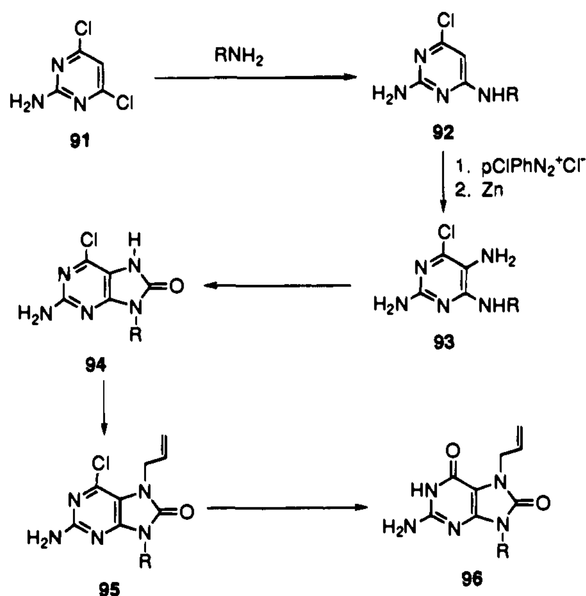
In addition to the ribose derivatives described above, we also prepared a number of compounds in which the ribose ring was radically altered or removed altogether. In the first such analogue, the ribose ring of **37** was replaced with a β-D-glucosyl moiety to construct molecule **81** (see Scheme 8). The synthesis of this compound started with N^9-(β-D-glucosyl)guanine (**77**), prepared by the method of Garner and co-workers.[28] Glucose derivative **77** was converted to **78** by the action of bromine. Treatment of this material with the anion of allyl alcohol resulted in decomposition under a variety of conditions, so we targeted the 8-thio analogue instead. 8-Mercapto analogue **79** was prepared by treatment of **78** with thiourea. Reaction of **79** with allyl bromide to give **80**, followed by thermal rearrangement at 130 °C in DMF afforded the target **81**, purified by reversed-phase HPLC.

Loxoribine was also treated with $NaIO_4$ to give dialdehyde **82**, which could be manipulated in the laboratory but was unstable to prolonged storage (Scheme 9). Reduction of **82** with $NaBH_4$ gave triol **83**. In addition, a double reductive amination[29] of **82** by reaction of butylamine or benzhydrylamine using $NaBH_3CN$ resulted in the preparation of morpholines **84** and **85**, respectively. 2-[(Hydroxyethoxy)methyl] derivative **90** was prepared as shown in Scheme 10. Acyclovir (**86**) was converted to 8-bromoacyclovir (**87**)[30] by treatment with bromine in water. In this case, direct reaction with

Scheme 10



Scheme 11



the anion of allyl alcohol under numerous conditions did not result in the addition of the allyl alcohol; rather, intramolecular reaction of the free hydroxyl on **87** occurred yielding **88**. Attempts to overcome this side reaction by protecting the free hydroxyl of **87** with silyl protecting groups were unsuccessful. Compound **88** was evaluated in our biological assays, and we redirected the synthesis to 8-thio derivative **90**. 8-Mercaptoacyclovir (**89**) was prepared from **87** and was then reacted with allyl bromide and subjected to the 3,3 rearrangement originally used in the preparation of **37**, to afford **90**.

Replacement of the ribose ring with several alkyl and aralkyl substituents was carried out as shown in Scheme 11.[31] This type of modification for **3** has recently been reported to yield compounds with antiviral activity against Semliki Forest virus infection in mice.[32] The most convenient manner for the construction of targets **97-104** (see Table 4) was by displacement of **91** with a primary amine to give **92**.[32] This was then followed by insertion of an amino group into the 6-position of the pyrimidine ring by diazotization and reduction of the diazo species which formed. Closure of the diamino functionality with ethyl chloroformate or phosgene gave urea **94**, which was allylated to afford **95**. The remaining chloride on the pyrimidine ring was subsequently hydrolyzed under acidic or basic conditions to give **96**. Product **103** is racemic, but the amine used to prepare **104** was obtained by the method of Huryn and co-workers and is enantiomerically pure [C1-(*R*)-C3-(*R*)].[33]

Table 4. 7,8,9-Trisubstituted Guanine Derivatives

compd	R	mp (°C)	formula
97	Bu	187−189	$C_{12}H_{17}N_5O_2{\cdot}0.25H_2O$[a]
98	$HO(CH_2)_4$	207−211	$C_{12}H_{17}N_5O_3$[b]
99	$PhCH_2$	>230	$C_{15}H_{15}N_5O_2$[c]
100	$(PhCH_2OCH_2)_2CH(CH_2)_2$	126−127	$C_{27}H_{31}N_5O_4$[d]
101	3,4-$(MeO)_2Ph(CH_2)_2$	>230	$C_{18}H_{21}N_5O_4$
102	C_8H_{17}	178−179	$C_{16}H_{25}N_5O_2$[e]
103	$HOCH_2$-cyclopentyl	201−203	$C_{14}H_{19}N_5O_3$
104	$HOCH_2$-tetrahydrofuranyl	187−189	$C_{13}H_{17}N_5O_4{\cdot}HCl{\cdot}0.5H_2O$[f]

[a] Recrystallization solvent was MeOH/ether. [b] Recrystallization solvent was EtOH. [c] Recrystallization solvent was iPrOH. [d] Recrystallization solvent was iPrOH. C: calcd, 66.24; found, 65.84. [e] Recrystallization solvent was EtOAc. [f] Recrystallization solvent was MeOH/ether. N: calcd, 19.85; found, 18.94.

Pharmacological Methods

The target guanosine derivatives were tested for their ability to enhance the immune system in three in vitro models,[34] and the results of these experiments are given in Table 5. The subject compounds were evaluated at the same time as a standard (internal control), and the data are reported both in terms of an ED_{50}, which can vary considerably (± ca. 50%), and the maximal response relative to the control. Loxoribine (**6**) was chosen early on as the lead compound in the series, so it was the standard used in most cases. However, other reference compounds were used in a few instances, and these exceptions are appropriately indicated.

The compounds were evaluated for their ability to induce a proliferative response in cultured spleen cells by measuring new DNA production via a [^{3}H]thymidine incorporation assay. The responding cells have previously been shown to be B (and not T) cells.[8a,34a]

The analogs were also tested in an adjuvanticity assay in which mouse spleen cells were incubated with sheep red blood cells (SRBC) as the antigen in the presence of incremental concentrations of drug. This test evaluates a compound's ability to augment the magnitude of an antibody response of primary B cells to respond to an antigen challenge.

In addition, activation of murine NK cell activity against Yac-1 lymphoma cells was measured.[34b] Spleen cells prepared from normal C3H/HeT mice were cultured for 18 h with different concentrations of the analogues, and then the cultured cells were tested for the ability to lyse Yac-1 cells in a 14-h ^{51}Cr-release assay. This test is in part a measure of the upregulation of cytokines such as interferon-γ and tumor necrosis factor (TNF).[35]

Several of the test compounds were also evaluated for in vivo NK cell activation. Mice were injected intravenously (iv), subcutaneously (sc), and orally (po) with the analogs, and 48 h later the spleens were removed and tested for the ability to lyse Yac-1 cells in a 4-h ^{51}Cr-release assay.

Structure−Activity Relationships

Most of the compounds in Table 5 were tested in two or more of the primary in vitro screens listed above; on

Table 5. In Vitro Immunostimulant Activity of the Target Compounds Relative to Loxoribine (**6**)

	mitogenicity		adjuvanticity		NK assay			mitogenicity		adjuvanticity		NK assay	
compd[a]	ED_{50} (μM)	% max resp (cmpd/stand.)	ED_{50} (μM)	% max resp (cmpd/stand.)	ED_{50} (μM)	% max resp (cmpd/stand.)	compd[a]	ED_{50} (μM)	% max resp (cmpd/stand.)	ED_{50} (μM)	% max resp (cmpd/stand.)	ED_{50} (μM)	% max resp (cmpd/stand.)
2	120	100[b]	10	99[c]	56, 94	72, 83	**47**	neg	0	47	77[c]	NT	NT
5	NC	82	15	82	NT	NT	**48**	NC	38	4	123	11	56
6	10, 50	100	3, 20	100	13, 22	100	**49**	neg	0	139	44	150, 220	19, 20
7	100	70	11	86	NT	NT	**50**	NC	NC	NC	NC	NT	NT
8	600	11	NC	NC	NT	NT	**51**	NT	NT	NT	NT	neg	0
9	neg	0	neg	0	NT	NT	**52**	neg	0	neg	9	neg	0
10	4, 500	5	103	73	NT	NT	**53**	NT[e]	NT	NT	NT	NT	NT
11	NT	NT	6	110	NT	NT	**54**	47	97	3	50	20, 27	90, 102
12	2, 400	14	135	44	NT	NT	**55**	NT	NT	1	5	neg	6
13	NT	NT	14	15	180	27	**56**	NT	NT	18	33	neg	15
14	50, 90	16, 28[b]	40	147, 187	48, 52	50, 59	**57**	82	57	15	76	neg	15
15	NT	NT	8	5	neg	6	**59**	neg	0.1	neg	1	neg	0
16	neg	0	neg	55	110, 150	15, 53	**60**	NT	NT	neg	0	NT	NT
17	NT	NT	3	16	neg	11	**61**	NT	NT	156	85	NT	NT
18	NT	NT	15	50	neg	0	**63**	30	84	6	45	11, 12	89, 99
19	neg	0	1, 620	23	NT	NT	**64**	52, 96	84, 127	6, 13	118, 192	14, 20	102, 104
20	630	58	48	86	NT	NT	**65**	neg	4	neg	19	neg	0
21	117	72	190	32	90, 100	42, 81	**66**	80	11	154	100	neg	0
22	820	63	194	83	NT	NT	**67**	NT	NT	NT	NT	NT	NT
23	neg	0	neg	0	NT	NT	**70**	233	76	17	143	60, 69	68, 109
24	NT	NT	4, 16	101, 139	19	83, 88	**73**	134	131	30	99	100	75
25	NT	NT	45	136	NT	NT	**74**	52	4	NC	32	NT	NT
26	NT	NT	142	12	NT	NT	**75**	neg	0	neg	9	neg	6
27	NT	NT	NC	3	NT	NT	**76**	NT	NT	neg	0[b]	NT	NT
28	NT	NT	NC	16	NT	NT	**81**	NT	NT	1	5	neg	3
29	NT	NT	39	91	NT	NT	**83**	37, 114	6	neg	0	NT	NT
30	NC	NC	28	110	NT	NT	**84**	49, 71	52, 96	5	38	17	94
34	neg	6	neg	8	neg	0	**85**	NT	NT	NT	NT	12	46
35	532	100[d]	50	100[d]	NT	NT	**88**	NT	NT	neg	0	neg	6
36	485	57[d]	107	107[d]	NT	NT	**89**	NT	NT	neg	0	3	26
37	20	151[b]	1	100[b]	NT	NT	**90**	13	7	14	9	55, 90	28, 92
38	118	107[c]	15	70[c]	NT	NT	**97**	11, 18	36, 40	2, 16	77, 168	4, 7	21, 43
39	34	96	5	141	NT	NT	**98**	52	16	neg	12	NT	NT
40	295	134	7	60	5	77, 86	**99**	NT	NT	5	25	NT	NT
41	NT	NT	53	109	neg	12	**100**	NT	NT	7	68	NT	NT
42	819	53	28	216	150	60	**101**	NT	NT	neg	0	NT	NT
43	NC	17[b]	168	193	NT	NT	**102**	NT	NT	37	130	NT	NT
44	45	9	55	89	40, 50	30, 36	**103**	neg	0	226	26	NT	NT
45	NC	10[b]	NC	16	NT	NT	**104**	272	16	83	10	neg	0
46	50	20	50	141, 265	30, 50	30, 36							

[a] NT is not tested, and NC is not calculated. Neg indicates a response lower than vehicle control. Each compound was tested at least once, as described in the Experimental Section. Where they were tested in more than one experiment, the highest and lowest values obtained are given. [b] Standard employed was compound **2**. [c] Standard employed was compound **37**. [d] Standard employed was compound **35**. [e] Compound was insoluble in the test assays.

the basis of the results of these tests, they were considered to fall into four broad categories. First are structures which display high activity in all three assays; second are those with selectivity for the adjuvanticity assay; third are those with selectivity for the mitogenicity and NK assays; and fourth are those which are weakly active or inactive in all of the assays.

Compounds Active in All Three Assays. Compounds with a medium alkyl chain (2-3 C) on the N-7 position of the purine ring (e.g., **5-7**) are consistently active. They are more active than the methyl derivative (**2**), and this activity is lost for the hexyl and decyl analogues (**8** and **9**). The 2-chloroethyl compound (**24**) is also active. Loxoribine, the allyl derivative (**6**), is particularly potent. Removal or modification of the 2′,3′-hydroxyls afforded a dideoxy analogue (**64**) related to loxoribine which exhibits comparable biological activity. 2′-Deoxy analogue **73** and the arabinose analogue **70** are also somewhat active, although not to the same degree as **64**. These results indicate that there is considerable tolerance with respect to allowable modifications in the sugar portion of the molecule. In addition, the solubility properties of **64** (see above) are considerably improved relative to loxoribine. 2′,3′-Ketal **48** displays considerable activity as well. The inactivity of **52**, which bears the polar amide functionality, also points to a hydrophobic binding region as being essential for activity of ketal derivatives. It is clear from the activity **64** of that free hydroxyls in the 2′- and 3′-positions are not essential.

Moderate activity in all three assays is also displayed by some of the peracylated derivatives (**39**, **40**, **42**), possibly because they act as prodrugs capable of being hydrolyzed in the cell to the parent nucleosides. The N-7 acetamide derivative (**21**) shows modest activity in these tests. Surprisingly, 5′-phosphate compound **57** also has moderate activity except in the NK assay. This activity could be the result of the hydrolysis of the 5′-phosphate group by 5′-nucleotidase-mediated dephosphorylation. Cottam and co-workers observed a similar result in evaluating the antiviral activity of the 5′-phosphate of **3**.[36] Replacement of the C-8 oxygen with sulfur or selenium atoms results in retention of activity within a given structural class. Increasing the size of

the sulfur-containing substituent on the C-8 position lessens activity; for example, the mitogenic activity of **36** was 57% that of **35**. Other compounds with larger groups (C-8 crotonylthio or cinnamylthio congeners, data not shown) have a substantially dimished effect.

Selectivity in the Adjuvanticity Assay. Certain of the N-7 benzyl-substituted derivatives, such as 4-nitrobenzyl analog **14**, are somewhat selective. Compound **14** displays fair potency in the adjuvanticity assay (0.04 mM), but has excellent efficacy compared to **6** (147–187%). The maximal response for this compound in the mitogenicity and NK assays falls in the range of 16–59% when compared to **6**. The N-9 alkyl and aralkyl analogues, lacking a ribose ring entirely, tend to also be selective adjuvants. The N-9 butyl analogue (**97**) is an example of such a compound. The activity of this compound when tested in a primary human culture system for the adjuvanticity effect, unlike that of **6**, decreases by ca. 10-fold, indicating species variability.[37] Several other compounds display selectivity for the adjuvanticity test, such as compounds **44** and **46**, which are isopropylidene ketals with a sulfur atom on C-8.

Selectivity for the Mitogenicity and NK Assays. Generally, activity in these two tests run in parallel; compounds active in one are active in the other. This suggests a common mechanism for both, perhaps through requirement for binding to the same intracellular proteins (receptors). Unsaturated dideoxy analog **63** and morpholine derivative **84** are selective for the mitogenic and NK responses. The activity of **84** is particularly impressive (e.g., ED_{50} of 17 μM with a maximum response of 94% relative to **6**), considering the radical modification of the sugar ring which has occurred. In addition, acyclovir derivative **90** appears to be selective for activity solely in the NK assay.

Compounds Inactive in All Three Assays. Compounds with large N-7 (e.g., **26**) or N-9 substituents (e.g., **103**) are inactive. Structures which have N-7 amine functionality, such as compounds **22**, **23**, **27**, **28**, and **30**, are inactive in the tests in which they were examined as well. The changes applied to the pyrimidine portion of the purine ring eliminated activity, such as with compound **59** and anhydro derivative **75**. The inactivity of these compounds could be attributed to a variety of causes, since **59** bears an amine N-7 substituent and **75** has had the 5′-hydroxyl removed. The 5′-deoxy derivative (**76**) and ketal **52** were inactive. Certain of the drastic sugar modifications in which hydroxyls were oriented in completely different arrangements than those in **6** were inactive, such as glucose analogue **81**, seco compound **83**, and α sugar **34**.

We do not know what the relative advantage would be to achieving selectivity in one or more of the tests described above. For compounds active in all three tests, the concentrations required for adjuvanticity and NK stimulation are about 1 order of magnitude lower that those needed for the mitogenicity assay. It could be that these immunostimulants are interacting with two or more distinct intracellular proteins (receptors), one of which has a higher affinity for the compounds being studied and is responsible for the adjuvanticity and NK effects.[38] There may be therapeutic benefit in selectively activating only one of the immunopotentiation pathways.

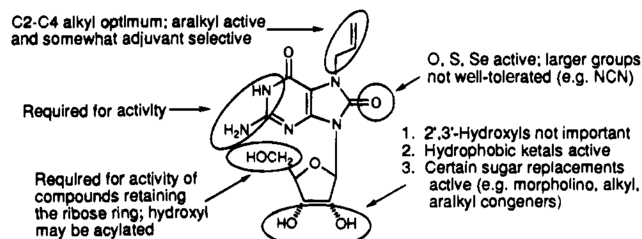


Figure 1. SAR of immunostimulants related to loxoribine.

A brief summary of our SAR work is given in Figure 1, which highlights key areas of the loxoribine structure. Two prominent features which stand out are the size dependence of the substituent on N-7 and the extreme extent to which the ribose ring can be modified.

In Vivo NK Activity. A selected subset of the compounds were evaluated for the ability to stimulate a murine NK response in vivo (48 h). After treatment with the test compound, mice were sacrificed and their splenocytes were examined for the ability to kill the Yac-1 target cell line. As shown in Table 6, loxoribine (**6**) and several other compounds displayed significant in vivo activity. The data for 2′,3′-dideoxy analogue **64** is particularly noteworthy because the compound is much more active than **6** upon oral administration.

Relevance to Natural Immunopotentiation. 8-Oxoguanosine residues play an important role in certain t-RNAs, promoting unusual conformations and turns.[39] Further, 8-oxopurine nucleosides (derived from adenosine and guanosine) are the product of ionizing radiation on DNA and RNA, causing a low incidence of mutation upon replication.[39,40] Compound **2** is produced naturally, being detected in small amounts in human urine, possibly as a result of a clearance pathway for 8-oxoguanosines.[41] It is possible that **2**, loxoribine, and other 8-substituted nucleoside immunostimulants may be activating a natural immunopotentiation scheme, ready to respond to the effects of damaged DNA and RNA.

Conclusions

Approximately 80 compounds were evaluated as immunostimulants, and they fall into four general categories: those with activity in all of the screens, those which have some selectivity for the adjuvant effect, those with primarily mitogenic and NK activity, and those which are inactive. A moderate-sized alkyl chain (2-4 C) on N-7 was optimal for activity. Oxo, thio, or seleno atoms on C-8 were allowed, but the rest of the purine ring could not be altered. In addition, there was considerable latitude in the structural modifications tolerated in the sugar portion of the molecule. The 2′- and 3′-hydroxyls could be removed altogether (such as in analog **64**), which also enhanced solubility properties in water and organic solvents. Certain other sugar modified analogues were active, including some of ketals involving the 2′,3′-hydroxyls, the morpholino derivatives, and the 5′-phosphate. Other drastic sugar modifications varying the nature or orientation of the hydroxyl groups were not allowed, such as for the glucose (**81**), 5′-deoxy (**76**), and seco (**83**) derivatives. When the sugar ring was replaced with alkyl or aralkyl substituents, the compounds were often active as well,

Table 6. In Vivo Enhancement of NK Activity by Guanosine Analogs

compd no.[a]	route	% lysis of YAC-1 cells at effector:target ratios				NK assay[b]	
		200:1	100	50:1	25:1	ED_{50} (μM)	% max resp (cmpd/stand.)
sesame oil	sc	7.8(1.0)	6.7(1.1)	4.2(0.3)	2.2(0.3)	–	–
6	sc	27.6(2.6)	19.5(2.0)	12.7(1.5)	9.3(1.1)	13, 22	100
14	sc	27.4(5.6)	20.7(5.4)	15.8(4.2)	9.9(2.7)	48, 52	50, 59
saline	iv	7.5(0.5)	7.1(1.1)	4.4(0.6)	2.4(0.1)	–	–
6	iv	52.2(1.0)	48.9(2.5)	38.6(2.1)	22.8(1.5)	13, 22	100
63	iv	47.4(2.0)	36.4(0.8)	25.7(0.4)	16.1(1.1)	11, 12	89, 99
64	iv	56.7(1.2)	42.8(2.2)	31.1(0.9)	18.4(1.8)	14, 20	102, 104
84	iv	43.9(0.8)	37.3(2.0)	27.6(2.3)	17.7(0.7)	17	94
saline	po	22.3(2.5)	13.9(1.8)	9.0(1.3)	6.0(0.6)	–	–
6	po	29.6(3.0)	18.9(2.2)	11.8(1.4)	7.7(0.4)	13, 22	100
63	po	50.8(2.8)	33.2(0.9)	25.2(0.4)	15.7(0.9)	11, 12	89, 99
64	po	40.5(4.6)	29.0(3.4)	19.2(2.7)	12.3(1.7)	14, 20	102, 104

[a] Mice were injected with 2 mg of compound. Spleens were removed after 48 h, and splenocytes were assayed for NK activity at four effector:target ratios. Results are expressed as the mean % lysis (± one standard deviation) of 5 × 104 YAC-1 target cells (N = 3–5 mice per group). Sc is subcutaneous; iv is intravenous; and po is parenteral (oral). [b] NK assay data is taken from Table 5 and is shown here for comparison.

although this was restricted to murine (not human) in vitro assays for **97**.

On the basis of a high level of activity in adjuvanticity and mitogenicity screens,[42] loxoribine (**6**) was chosen for further evaluation in other preclinical models and for development as a clinical candidate. It enhances murine NK activity in a dose- and time-dependent fashion in spleen, bone marrow, and blood.[43]

Experimental Section

General Procedures. ^{1}H NMR spectra were recorded on either a Bruker AC-300 (300 MHz), AM-360WB (360 MHz), Bruker AM-400 (400 MHz), or Varian 390 (90 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AM-360WB (100 MHz) spectrometer. For NMR work, DMSO-d_6 was used as the solvent unless otherwise noted, and tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were mainly conducted by the Analytical Services group at Raritan, NJ; those samples requiring water analysis were evaluated by Robertson Microlit, Madison, NJ. Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are corrected. Infrared spectra were recorded on a Perkin-Elmer 1420 IR spectrometer. Chemical-ionization mass spectra (CI-MS) were recorded on a Finnigan 3300-6100 system with methane as the reagent gas unless otherwise noted. Fast-atom-bombardment mass spectra (FAB-MS) were obtained on a VG 7070E spectrometer. An Ion Tech saddlefield gun, which generated a primary beam of argon atoms at 8 keV and 2 mA was used for the FAB analysis. Where elemental analyses are reported by symbols of elements, the results are within 0.4% of the calculated values. Most reagents and solvents were purchased as appropriate and used without further purification.

General Synthesis of 7-Alkyl-2-amino-9-β-D-ribofuranosylpurine-6,8(1*H*)-diones (5-24). The syntheses of 2-amino-7-(4-nitrobenzyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione (**14**) and 2-amino-7-acetamido-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione (**21**) are typical procedures for the preparation of these compounds and are given below.

2-Amino-7-(4-nitrobenzyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hemihydrate (14). To a mixture of sodium hydride (60% oil dispersion, 1.30 g, 32.5 mM) and DMF (300 mL) was added **31** (10 g, 31.84 mM) all at once at 0 °C under nitrogen, and the resulting solution was stirred for 10 min. To this was added 4-nitrobenzyl bromide (10.8 g, 50.0 mM), and the resulting mixture was allowed to warm to room temperature and stirred for 30 min. Most of the DMF was removed in vacuo, and the brown residue was treated with ethyl acetate (250 mL) and water (300 mL). The mixture was stirred for 16 h, and the yellow crystals were filtered, washed with water, acetone, and ether, and then dried in vacuo at 60 °C overnight to give 1,2-diamino-7-(4-nitrobenzyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione (11.9 g, 83%) as a yellow powder. This was used in the following reaction without further purification. To a solution of this material (6 g, 13.36 mM) and concentrated HCl (5 mL) in DMF (400 mL) was added a solution of sodium nitrite (1 g, 15.94 mM) in water (20 mL) at 0 °C, and the resulting solution was stirred for 30 min. Sodium acetate (5 g, 60.98 mM) was added, followed by stirring for 5 min. Most of the solvent was removed under vacuum, and the brown residue was purified by column chromatography on silica gel (300 g, CH_3OH/CH_2Cl_2, 1:9) to give **14** as a yellow powder (4.2 g, 71%), mp 265–267 °C dec. Compounds of this type were analytically pure after chromatography and were generally not recrystallized: IR (KBr) 1715, 1679, 1643, 1611, 1589, 1523 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.4 (bs, 1H, exchangeable, NH), 8.2 (d, J = 8 Hz, 2H), 7.5 (d, J = 8 Hz, 2H), 6.7 (bs, 2H, exchangeable, NH_2), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$), 5.1 (s, 2H, benzylic protons); ^{13}C NMR (DMSO-d_6) δ 154.1, 152.6, 150.9, 146.9, 146.8, 145.5, 128.6, 123.7, 98.5, 85.8, 85.1, 70.7, 69.9, 62.2, 44.0. CI-MS m/e 435 (MH$^+$). Anal. ($C_{17}H_{18}N_6O_8 \cdot 0.5H_2O$) C, H, N.

2-Amino-7-acetamido-9-β-D-ribofuranosylpurine-6,8-(1*H*)-dione Hemihydrate (21). To a mixture of sodium hydride (60% oil dispersion, 1 g, 25 mM) and DMF (300 mL) was added **33** (5 g, 15.9 mM) and MeOH (1 mL) at 0 °C under nitrogen, and the resulting solution was stirred for 10 min. Bromoacetamide (3.2 g, 23.4 mM) was then added, and the resulting mixture was allowed to warm to room temperature and stirred for 16 h. Most of the DMF was removed in vacuo, and the residue was treated with water (250 mL) and extracted with ethyl acetate (2 × 100 mL). The aqueous layer was concentrated to ca. 50 mL and allowed to sit overnight. The white crystals which formed were filtered, washed with acetone, and then dried in a vacuum oven overnight at 60 °C to give 1,2-diamino-7-acetamido-9-β-D-ribofuranosylpurine-6,8-(1*H*)-dione as white crystals (4.5 g, 72%): mp 261–264 °C dec; IR (KBr) 1676, 1647, 1635, 1597, cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.48 (bs, 1H, exchangeable, CONH), 7.12 (bs, 2H, exchangeable, NH_2), 7.08 (bs, 1H, exchangeable, CONH), 5.61 (d, J = 6 Hz, 1H, $C_{1'}$), 5.37 (bs, 2H, exchangeable, NNH_2), 4.39 (s, 2H, CH_2CO); ^{13}C NMR (DMSO-d_6) δ 168.8, 153.9, 151.3, 151.2, 143.7, 98.7, 85.7, 84.8, 70.7, 70.0, 62.3, 43.3. Anal. ($C_{12}H_{17}N_7O_7$) C, H, N. To a solution of this material (1.5 g, 4.04 mM) and concentrated HCl (0.3 mL) in DMF (100 mL) was added a solution of sodium nitrite (310 mg, 4.49 mM) in water (5 mL) at 0 °C, and the mixture was stirred for 30 min. Sodium acetate (1 g) was added, and the resulting mixture was stirred for 5 min. Most of the solvent was removed in vacuo, and the residue was treated with water (30 mL) and stirred overnight. The white precipitate which emerged was filtered, washed with cold water and acetone, and then dried in a vacuum oven at 60 °C overnight to give **21** as an off-white powder (1.15 g, 77%): mp 245–247 °C dec; IR (KBr) 1699, 1654, 1625, 1599, cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.86 (bs, 1H, exchangeable, NH), 7.47 (bs, 1H, exchangeable, CONH), 7.09 (bs, 1H, exchangeable, CONH), 6.47 (bs, 2H,

exchangeable, NH_2), 5.61 (d, J = 6 Hz, 1H, $C_{1'}$), 4.36 (s, 2H, CH_2CO); ^{13}C NMR (DMSO-d_6) δ 168.8, 153.1, 151.6, 151.2, 146.2, 99.5, 85.9, 84.9, 70.7, 70.1, 62.3, 43.5. Anal. ($C_{12}H_{16}N_6O_7$·0.5H_2O) C, H, N.

2-Amino-7-[2-(4-chlorophenyl)-2-oxoethyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione (19). This compound was prepared from **31** in 35% yield by the same sequence as described for **14** and **21**: mp 151–153 °C; IR (KBr) 1700, 1680, 1630 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.70 (bs, 1H, exchangeable, CONH), 8.11 (d, J = 10 Hz, 2H), 7.70 (d, J = 10 Hz, 2H), 6.5 (bs, 2H, exchangeable, NH_2), 5.60 (d, J = 6 Hz, 1H, $C_{1'}$), 5.30 (s, 2H). Anal. ($C_{18}H_{18}ClN_5O_7$) C, H, N.

2-Amino-7-[3-(dimethylamino)propyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hydrochloride Dihydrate (22). The title compound was prepared from **31** as described above: mp 180 °C dec; IR (KBr) 1670, 1620, 1590 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.7 (bs, 1H, exchangeable, NH), 6.9 (bs, 2H, exchangeable, NH_2), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$), 2.7 (s, 6H). Anal. ($C_{15}H_{24}N_6O_6$·HCl·2H_2O) C, H, N.

2-Amino-7-[2-(1-piperidino)ethyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hydrochloride Hydrate (23). This title compound was prepared as described above: mp 157 °C dec; IR (KBr) 1700, 1670, 1620, 1590 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 11.2 (bs, 1H, exchangeable), 9.97 (bs, 1H, exchangeable, NH), 6.9 (bs, 2H, exchangeable, NH_2), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{17}H_{26}N_6O_6$·HCl·H_2O) C, H, N.

1,2-Diamino-7-(2,3-epoxypropyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione (32). To a solution of **31** (572 mg, 1.82 mM) in DMF (40 mL) was added NaH (60% oil dispersion, 75 mg, 1.88 mM) at room temperature under a nitrogen atmosphere, and the mixture was stirred for 5 min. To this solution was added epibromohydrin (0.5 mL, 5.83 mM), and the resulting mixture was stirred overnight. Workup as described for **14** and **21** gave a crude sample of **32**, which decomposed slowly upon long-term storage at room temperature. This crude product was used directly in the next step without further purification.

2-Amino-7-(2,3-dihydroxypropyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hydrate (25). The title compound was obtained as a white powder in 20% overall yield by purification by reversed-phase HPLC (water) of crude **32**: mp 195–196 °C dec; IR (KBr) 1680, 1660, 1640 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.7 (bs, 1H, exchangeable, NH), 6.5 (bs, 2H, exchangeable, NH_2), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{13}H_{19}N_5O_8$·H_2O) C, H, N.

2-Amino-7-[2-hydroxy-3-(phenylthio)propyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hemihydrate (26). A mixture of crude **32** (3 g, 5.4 mM) prepared as described above and thiophenol (5 g, 45.4 mM) in DMF (150 mL) was heated at 80 °C under nitrogen for 4 h. Most of the solvent was removed in vacuo, and the residue was dissolved in water (500 mL). The product was purified by preparative HPLC (C-18 reverse-phase column, MeOH/water, 2:8–3:7) to give 1,2-diamino-7-[2-hydroxy-3-(phenylthio)propyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione as an off-white powder (52%): mp 135–137 °C dec; IR (KBr) 1700, 1580, 1100 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.25 (m, 5H, aromatic protons), 7.15 (bs, 2H, exchangeable, NH_2), 5.65 (d, J = 5 Hz, 1H, $C_{1'}$), 5.35 (bs, 2H). Anal. ($C_{19}H_{24}N_6O_7S$) C, H, N. Deamination of this material was carried out the same way as described for the preparation of **14** and **21** to give **26** as a light brown powder (42%): mp 180–182 °C dec; IR (KBr) 1690, 1635, 1600, 1100 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.7 (bs, 1H, exchangeable, NH), 7.15 (m, 5H), 6.3 (bs, 2H, exchangeable, NH_2), 5.7 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{19}H_{23}N_5O_7S$·0.5 H_2O) C, H, N.

2-Amino-7-[3-[(3,4-dimethoxyphenethyl)amino]-2-(hydroxy)propyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hydrochloride Hydrate (27). The title compound was prepared from **32** and 3,4-dimethoxyphenethylamine in 20% overall yield: mp 163–170 °C dec; IR (KBr) 1680, 1640, 1600, 1030 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 8.9 (bs, 1H, exchangeable, NH), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$), 5.59 and 5.62 (both s, 3H each, CH_3O). Anal. ($C_{23}H_{32}N_6O_9$·HCl·H_2O) C, H, N.

2-Amino-7-[3-[1-[4-(4-fluorophenyl)piperazinyl]]-2-hydroxypropyl]-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hemihydrate (28). The title compound was prepared from **32** and *N*-(4-fluorophenyl)piperazine in 25% overall yield: mp 178–181 °C dec; IR (KBr) 1690, 1660, 1390 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.7 (bs, 1H, exchangeable, NH), 6.8–7.1 (m, 4H), 6.3 (bs, 2H, exchangeable, NH_2), 5.2 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{23}H_{30}FN_7O_7$·0.5H_2O) C, H, N.

2-Amino-7-(3-azido-2-hydroxypropyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Hydrate (29). The title compound was obtained as a white powder in 12% overall yield from the crude **32** and sodium azide: mp 138–141 °C dec; IR (KBr) 2110, 1690, 1660 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.7 (bs, 1H, exchangeable, NH), 6.4 (bs, 2H, exchangeable, NH_2), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{13}H_{18}N_8O_7$·H_2O) C, H, N.

2-Amino-7-(3-amino-2-hydroxypropyl)-9-β-D-ribofuranosylpurine-6,8(1*H*)-dione Formate 0.75Hydrate (30). A mixture of **29** (150 mg), ammonium formate (130 mg), 10% Pd/C (6 mg), methanol (10 mL), and water (20 mL) was heated to reflux under nitrogen for 16 h. The resulting mixture was cooled to room temperature, filtered through a pad of Celite, and washed with water (10 mL). The filtrate was concentrated in vacuo, and the residue was dissolved in water (5 mL). This was purified by preparative HPLC (C-18 reverse-phase column, MeOH/water, 1:9) to give **32** (86 mg, 55%) as a white powder; mp 150 °C dec; IR (KBr) 2110, 1690, 1660 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.7 (bs, 1H, exchangeable, NH), 6.4 (bs, 2H, exchangeable, NH_2), 5.6 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{13}H_{20}N_6O_7$·CH_2O_2·0.75H_2O) C, H, N.

2-Amino-8-thioxo-9-α-D-ribofuranosylpurin-6(1*H*)-one Hemihydrate (34). A solution of 2-amino-8-thioxo-9-[2′,3′-isopropylidene-5′-(*tert*-butyldimethylsilyl)-α-D-ribofuranosyl]purin-6(1*H*)-one[16b] (1.50 g, 3.4 mmol) and freshly-recrystallized *N*-bromosuccinimide (1.22 g, 6.8 mmol) in $CHCl_3$ (30 mL) was allowed to stir at room temperature. After 5 h, the solvent was removed and the residue dissolved in ether and washed twice with water and once with brine. The organic layer was treated with CH_2Cl_2 to prevent the 8-bromo product from precipitating, dried ($MgSO_4$), filtered, and concentrated to give 2-amino-8-bromo-9-[2′,3′-isopropylidene-5′-(*tert*-butyldimethylsilyl)-α-D-ribofuranoyl]purin-6(1*H*)-one as an orange foam (ca. 2 g). This material was purified by flash column chromatography (CH_2Cl_2/MeOH, 93:7) which afforded the 8-bromo derivative as a yellow glass (1.0 g, 56%) whose ^1H NMR (400 MHz) was consistent with the structure. A solution of this glass (0.93 g, 1.8 mmol) and thiourea (1.37 g, 18 mmol) in BuOH (15 mL) was heated at reflux for a total of 12 h. The solution was cooled, water was added, and the solid which emerged overnight was washed with water and ether and air-dried to give 0.17 g (29%) of **34** as a gray powder. Inspection of the 300-MHz ^1H NMR at this point revealed that the silyl and isopropylidene groups had been removed during the course of the reaction. This powder was recrystallized by dissolving in a 3:1 mixture of DMF/DMSO (2 mL), filtering, and adding ca. 18 mL of MeOH. After scratching, a crystalline solid emerged which was filtered and collected (88 mg): mp 275–330 °C dec; IR (KBr) 1697, 1643, 1548, 1494, 1444, 1378 cm^{-1}; NMR (400-MHz, DMSO-d_6) δ 3.42–3.46 (m, 1H), 3.54–3.57 (m, 1H), 3.98 (t, J = 5.5 Hz), 4.30–4.35 (m, 2H), 4.5–5.1 (bs, 2H), 5.3–5.8 (bs, 1H), 6.59 (s, 2H), 6.72–6.74 (d, J = 6.6 Hz), 11.1 (s, 1H). The ^1H NMR revealed the presence of a small amount of DMF (ca. 0.1 molar equiv). Anal. ($C_{10}H_{13}N_5O_5S$·0.5H_2O·0.11DMF) C, H; N: calcd, 21.54; found, 21.03.

8-(2-Propenylthio)guanosine (36). To a mixture of 8-thioguanosine (20 g, 63.5 mM) and potassium carbonate (10 g, 72 mM) in DMF (300 mL) was added allyl bromide (8 g, 63.5 mM). The resulting mixture was heated at 45 °C for 90 min. The mixture was cooled to room temperature and poured into a mixture of ether (1.4 L) and acetic acid (5 mL) with stirring. The solid which formed was filtered, washed with cold water, acetone, and ether, and then dried in a vacuum oven at 60 °C overnight to give **36** as a white powder (14.7 g, 67%): mp 225 °C dec; IR (KBr) 1700, 1640, 1610 cm^{-1}; ^1H NMR (DMSO-d_6, 90 MHz) δ 10.86 (bs, 1H, exchangeable, NH), 6.47 (bs, 2H, exchangeable, NH_2), 5.61 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{13}H_{17}N_5O_5S$) C, H, N.

2-Amino-7-(2-propenyl)-9-β-D-ribofuranosylpurine-6-(1H)-one-8-thione (37). A solution of **36** (60 g, 169 mM) in DMF (700 mL) was heated at 130 °C under nitrogen for 9 d. The resulting mixture was cooled to room temperature, and the solvent was removed in vacuo. The residue was treated with MeOH (400 mL) with stirring. The solid was filtered and recrystallized from hot EtOH–water to give **37** as a white powder (33.5 g, 55%): mp >230 °C; IR (KBr) 1700, 1635, 1605, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.13 (bs, 1H, exchangeable, NH), 6.64 (bs, 2H, exchangeable, NH_2), 6.31 (d, J = 6 Hz, 1H, $C_{1'}$); ^{13}C NMR (DMSO-d_6) δ 166.21, 154.02, 151.93, 149.68, 132.41, 118.30, 104.89, 90.00, 85.51, 70.99, 70.89, 62.65, 47.83. Anal. ($C_{13}H_{17}N_5O_5S$) C, H, N.

2-Amino-6-oxo-7-(2-propenyl)-9-β-D-ribofuranosylpurin-8(1H)-selenone 0.25Hydrate (38). To a mixture of 8-selenoxoguanosine (6.0 g, 16.6 mM) and potassium carbonate (3.0 g, 21.7 mM) in DMF (100 mL) was added allyl bromide (2 g, 16.7 mM). The resulting mixture was stirred at 45 °C under nitrogen for 2 d. The mixture was cooled to room temperature, and the solvent was removed in vacuo. The residue was treated with ether (200 mL) with stirring. The solid was filtered, washed with ether, and then treated with water (150 mL) and acetic acid (5 mL). The solid was filtered and washed with cold water and acetone to give 2-amino-8-(2-propenylseleno)-9-β-D-ribofuranosylpurin-6-one as a yellow powder (3.85 g, 57%). A solution of this material (1.0 g, 2.5 mM) in DMF (100 mL) was stirred at 120 °C under nitrogen for 24 h. The resulting mixture was cooled to room temperature, and the solvent was removed in vacuo. The residue was treated with ether (150 mL) with stirring. The solid which formed was filtered and recrystallized from water to give **38** as a beige powder (220 mg, 22%): mp 227–230 °C dec; IR (KBr) 1690, 1640, 1620, 1590 cm^{-1}; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 11.02 (bs, 1H, exchangeable, NH), 6.58 (bs, 2H, exchangeable, NH_2), 6.37 (d, J = 6 Hz, 1H, $C_{1'}$). Anal. ($C_{13}H_{17}N_5O_5Se \cdot 0.25H_2O$) C, H, N.

2-(Acetylamino)-7-(2-propenyl)-8-thioxo-9-(2',3',5'-triacetyl-β-D-ribofuranosyl)purin-6(1H)-one (40). To a mixture of **37** (5 g, 14.0 mM), triethylamine (10 mL), acetic anhydride (6.0 mL, 65 mM), and methylene chloride (250 mL) was added 4-(N,N-dimethylamino)pyridine (25 mg) at room temperature under nitrogen. The solution was stirred overnight, diluted with methylene chloride (250 mL), washed with 1 N HCl, brine, and water, and dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (500 g, ethyl acetate/hexane, 5:1, v/v) to give **40** as an off-white powder (2.9 g, 40%): mp 90–92 °C; IR (KBr) 1750, 1690 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.12 (bs, 1H, exchangeable, NH), 11.41 (bs, 1H, exchangeable, NH), 6.43 (d, J = 6 Hz, 1H, $C_{1'}$) 2.15, 2.00, 1.94, 1.90 (each s, 3H each). Anal. ($C_{21}H_{25}N_5O_9S$) C, H, N.

2-Amino-7-(2-propenyl)-8-thioxo-9-(2',3',5'-triacetyl-β-D-ribofuranosyl)purin-6(1H)-one (39). Continued elution of the above column with ethyl acetate gave **39** as a pale yellow powder (1.5 g, 23%): mp 100–102 °C; IR (KBr) 1740, 1690, 1630, 1450, 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.77 (d, J = 6 Hz, 1H, $C_{1'}$), 2.01 (s, 6H), 1.96 (s, 3H). Anal. ($C_{19}H_{23}N_5O_8S$) C, H, N.

2-(Butanoylamino)-7-(2-propenyl)-9-(2',3',5'-tributanoyl-β-D-ribofuranosyl)purine-6,8(1H)-dione (41). A suspension of **6** (500 mg, 1.47 mmol) in acetonitrile (10 mL) was treated with butanoyl chloride (919 μL, 8.85 mmol), and the solution was heated at reflux for 4 h. Saturated aqueous sodium bicarbonate was added, and the product was extracted into methylene chloride. The organic layer was withdrawn, dried ($MgSO_4$), filtered, and concentrated. The target structure was purified on silica gel (CH_2Cl_2/MeOH, 93:7) to give **41** as a tacky white semisolid (450 mg, 50%): mp 81–83 °C; ^{1}H NMR ($CDCl_3$) δ 0.9–1.1 (m, 16H), 1.6–1.75 (m, 12H), 1.78 (m, 2H), 2.3 (m, 6H), 2.51 (t, 2H), 4.4 (m, 2H), 4.62 (d, 2H), 4.71 (m, 1H), 5.2 (dd, 2H), 5.81 (m, 1H), 6.0 (m, 2H), 6.12 (d, 1H), 9.12 (s, 1H), 11.99 (s, 1H); $[\alpha]^{25}_D$ −2.5° (c 1.0, MeOH). Anal. ($C_{29}H_{41}N_5O_{10}$) C, H, N.

2-[(2-Methoxyacetyl)amino]-7-(2-propenyl)-9-[2',3',5'-tris(2-methoxyacetyl)-β-D-ribofuranosyl]purine-6,8(1H)-dione (42). This compound was prepared in an analogous manner to the preparation of **41** from **6** and 2-methoxyacetyl chloride, yielding giving 310 mg of **42** (33%) as a stiff foam: MS (FAB thio) m/e 628 (MH^+); $[\alpha]^{25}_D$ −2.2° (c 1.0, MeOH). ^{1}H NMR ($CDCl_3$) δ 3.4–3.5 (m, 9H), 3.58 (s, 3H), 4.0–4.15 (m, 8H), 4.38 (m, 2H), 4.55 (m, 1H), 4.69 (m, 2H), 5.26 (dd, 2H), 5.81 (m, 1H), 6.0 (m, 3H), 6.20 (d, 1H), 9.31 (s, 1H), 11.8 (s, 1H). Anal. ($C_{25}H_{33}N_5O_{14}$) C, H, N.

2-Amino-7-methyl-9-(2',3'-isopropylidene-β-D-ribofuranosyl)purine-6,8(1H)-dione (43). The syntheses of **43-45** can be illustrated by that for **43**, given here. A mixture of 1 (1.5 g, 4.79 mM), 2,2-dimethoxypropane (1.8 mL, 14.7 mM), acetone (100 mL), and concentrated sulfuric acid (0.2 mL) was stirred at room temperature under nitrogen for 40 h. The resulting mixture was cooled to 0 °C and treated with concentrated ammonium hydroxide solution (5 mL). The solvent was removed in vacuo, and the solid was filtered. The solid was washed successively with water, acetone, and ether, and then dried in vacuo at 60 °C to give **43** (1.12 g, 65%) as a white powder: mp 193 °C dec; IR (KBr) 1680, 1650, 1610, 1460 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 3.31 (s, 3H, CH_3N) 1.52 and 1.31 (both s, 3H each, Me's). Anal. ($C_{14}H_{19}N_5O_6 \cdot H_2O$) C, H, N.

2-Amino-7-(2-propenyl)-8-thioxo-9-(2',3'-isopropylidene-5'-acetyl-β-D-ribofuranosyl)purin-6(1H)-one (46). A mixture of **44** (1.2 g, 3.0 mM), triethylamine (3 mL), acetic anhydride (0.3 g, 2.9 mM), and methylene chloride (100 mL) was stirred at room temperature for 16 h. The mixture was poured into water (100 mL), the organic layer was separated, and the aqueous layer was extracted with methylene chloride (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (100 g, EtOAc/hexane, 9:1 v/v) to give the title compound as an off-white powder (900 mg, 70%): mp 179 °C dec; IR (KBr) 1730, 1710, 1680, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 11.1 (bs, 1H, exchangeable, NH), 6.85 (bs, 2H, exchangeable, NH_2), 6.60 (s, 1H, $C_{1'}$), 2.01 (s, 3H, CH_3CO), 1.45 and 1.32 (both s, 3H each). Anal. ($C_{18}H_{23}N_5O_6S$) C, H, N.

2-Amino-7-(2-propenyl)-8-thioxo-9-(2',3'-isopropylidene-5'-benzoyl-β-D-ribofuranosyl)purin-6(1H)-one 0.25Hydrate (47). To a mixture of **44** (2.5 g, 6.3 mM) and triethylamine (5 mL) in methylene chloride (150 mL) was added benzoyl chloride (1.2 g, 8.5 mM) at 0 °C under nitrogen during a 10 min period. After addition, the mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was poured into water (100 mL), the organic layer was separated, and the aqueous layer was extracted with additional methylene chloride (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (300 g, ethyl acetate/hexane, 4:1, v/v) to give **47** as pale yellow powder (650 mg, 21%): mp 214 °C dec; IR (KBr) 1700, 1630, 1590, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.12 (bs, 1H, exchangeable, NH), 7.90–7.32 (m, 5H, phenyl H's), 6.73 (bs, 2H, NH_2), 6.61 (s, 1H, $C_{1'}$), 1.62 and 1.39 (both s, 3H each). Anal. ($C_{23}H_{25}N_5O_6S \cdot 0.25H_2O$) C, H, N.

Ketalization of Compound 6. The preparation of **48-50** is illustrated by the synthesis of **50**.

2-Amino-7-allyl-9-[2',3'-[4-(benzyloxycarbonyl)-2-butylidene]-β-D-ribofuranosyl]purine-6,8(1H)-dione (50). A mixture of **6** (21.0 g, 62 mmol), benzyl levulinate (25.6 g, 124 mmol), 7 N HCl/dioxane (30 mL, 210 mmol), and triethyl orthoformate (15.6 mL, 94 mmol) was stirred for 1 d at ambient temperature in DMF (225 mL). Dilution of the reaction with ether and hexane deposited a dark brown oil which was purified by silica gel chromatography ($CHCl_3$). Recrystallization ($CHCl_3$/hexane) provided ketal **50** (19.53 g, 60%) as a 84:16 mixture of endo:exo diastereomers based on integration of the methyl group protons:[19] mp 140–142 °C; FAB-MS m/e 528 (M + 1, 60); ^{1}H-NMR (DMSO-d_6) δ 1.25 (t, 2.52H), 1.45 (s, 0.48H), 2.05 (t, 2H), 3.5 (t, 2H), 4.0 (m, 1H), 4.4 (d, 2H), 4.8 (s, 1H, broad), 4.95-5.1 (m, 5H), 5.4 (d, 1H), 5.91 (m, 1H), 6.6 (s, 2H), 7.35 (m, 5H). Anal. ($C_{25}H_{29}N_5O_8 \cdot 0.3H_2O$) C, H, N, H_2O.

2-Amino-7-(2-propenyl)-9-[2',3'-(4-carboxy-2-butylidene)-β-D-ribofuranosyl]purine-6,8(1H)-dione 0.3Hydrate (51). A solution of **49** (1.0 mg, 2.1 mmol) and NaOH (26 mL of a 1

M aqueous solution) in EtOH (26 mL) was allowed to stir for 45 min at room temperature. Amberlite IR-50 ion-exchange resin (H^+) was added until the pH of the solution was in the range 5–6. The resin was filtered and then rinsed additionally with EtOH and water. The combined filtrates were evaporated under vacuum. Addition of water and warming resulted in crystallization of the residue. The resulting solid was recrystallized from water and then dried at room temperature under vacuum to give 0.46 g of pure **51** (49%): mp 239–241 °C; IR (KBr) 1683, 1638, 1598, 1448 cm^{-1}; $[\alpha]^{20}_D = -29.2°$ (*c* 1.0, DMSO); 300-MHz ^1H NMR in DMSO-d_6 was consistent with the structure as a 96:4 mixture of *endo*-Me:*exo*-Me diastereomers; MS (FAB) *m/e* 438 (M + 1). Anal. ($C_{18}H_{23}N_5O_8 \cdot 0.3H_2O$) C, H, H_2O; N: calcd, 15.79; found, 15.36.

2-Amino-7-(2-propenyl)-9-[2′,3′-[4-(N-methoxycarbamoyl)-2-butylidene]-β-D-ribofuranosyl]purine-6,8(1*H*)-dione 0.75Hydrate (52). A solution of **49** (0.6 g, 1.28 mmol) in MeOH (7 mL) was treated with methylamine for ca. 5 min while cooling in an ice/water bath. After stirring in a stoppered flask for 4 d, the solvents were removed under vacuum to give 0.62 g of a white foam. The foam was dissolved into a mixture of CH_2Cl_2 and MeOH, filtered through a filter aid, and concentrated to give a solid glass-like substance (**52**, 0.55 g, 95%): mp 142.5–147.5 °C; IR (KBr) 1680, 1635, 1598, 1435, 1386 cm^{-1}; $[\alpha]^{20}_D = -29.9°$ (*c* 1.0, MeOH); MS (FAB) *m/e* 451 (M + 1, 100); 300-MHz ^1H NMR in DMSO-d_6 revealed an 89:11 mixture of *endo*-Me:*exo*-Me diastereomers. Anal. ($C_{19}H_{26}N_6O_7 \cdot 0.75H_2O$) C, H, N, H_2O.

2-Amino-7-(2-propenyl)-9-[5′-(*tert*-butyldiphenylsilyl)-β-D-ribofuranosyl]purine-6,8(1*H*)-dione Hydrate (56). A solution of **7** (1.0 g, 2.96 mmol), imidazole (0.22 g, 3.26 mmol), and *tert*-butyldiphenylsilyl chloride (0.76 mL, 3.26 mmol) in pyridine (6 mL) was allowed to stir at ambient temperature. After 6 h, water (25 mL) was added to the reaction. After 30 min, the resultant white solid was collected and washed three times with ether. After drying under vacuum at 55 °C overnight, there was obtained 0.90 g of white solid (50%). High-field ^1H NMR and mass spectral analysis supported the assigned structure. Anal. ($C_{29}H_{34}N_5O_6Si \cdot H_2O$) C, H, N, H_2O.

2-Amino-7-(2-propenyl)-9-[2′,3′-[(4-fluorophenyl)methylidene]-β-D-ribofuranosyl]purine-6,8(1*H*)-dione (53). A solution of **55**[16a] (1.00 g, 1.73 mmol), 4-fluorobenzaldehyde (1.86 mL, 17.3 mmol), and H_2SO_4 (1 drop) in 15 mL of THF was allowed to stir at room temperature over the weekend. Triethylamine was added until the solution was basic, and the solvents were evaporated. The product was chromatographed on silica gel in 93:6:1 CH_2Cl_2/MeOH/NH_4OH to give fractions containing **53** as the 5′-*tert*-butyldiphenylsilyl ether (0.67 g, 57%): ^1H NMR (DMSO-d_6) δ 0.99 (s, 9H), 3.82 (m, 2H), 4.4 (br m, 3H), 5.0 (dd, 2H), 5.16 (br m, 1H), 5.43 (d, 1H), 5.82 (m, 1H), 6.0 (d, 2H), 6.58 (br s, 2H), 7.3–7.7 (m, 14 H). To a solution of this material (0.49 g, 0.72 mmol) in THF (5 mL) was added 1 M tetra-*n*-butylammonium fluoride (2 mL). After 5 h, the THF was evaporated and the residue was stirred in iPrOH. The crystals that formed were collected, washed with ether, recrystallized again from iPrOH, and dried to give the title compounds as a white powder (0.27 g, 84%). As there was still a trace amount of tetra-*n*-butylammonium fluoride observed by ^1H NMR, the sample was recrystallized from DMSO/water to give a freely flowing white powder (0.14 g, 44%; mp 311–314 °C, dec): CI-MS (NH_3) *m/e* 446 (MH^+); $[\alpha]^{25}_D$ −72.0 (*c* 0.35, DMSO). A single diastereomer could be seen in the 360-MHz ^1H NMR (DMSO-d_6), assigned as the exo isomer. Anal. ($C_{20}H_{20}FN_5O_6$) C, H, N.

2-Amino-7-(2-propenyl)-9-(2′,3′-carbonyl-β-D-ribofuranosyl)purine-6,8(1*H*)-dione 0.2Hydrate (54). A solution of **56** (1.28 g, 2.82 mmol) and 1,1-carbonyldiimidazole (0.91 g, 5.64 mmol) in dry THF (10 mL) was allowed to stir overnight under nitrogen atmosphere. Most of the THF was evaporated, and water was added. The product crystallized upon standing overnight. The crystals were filtered, washed with water and ether, and then air-dried to give 1.21 g (90%) of **54** with the 5′-silyl protective group. This was purified further by flash silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH, 90:9:1) to give 0.63 g (47%) of white crystalline powder. The majority of this material (0.52 g, 1.08 mmol) and tetra-*n*-butylammonium fluoride (2.1 mL of a 1 M solution in THF) were added to THF (10 mL), and the solution was allowed to stir at room temperature overnight. The THF was then largely removed, and the residue was treated with water. The crystals were collected and washed with water, ether, and 2-propanol and then air-dried to give 0.29 g (74%) of fluffy white crystals of **54** (mp 323–325 °C dec). High-field ^1H NMR and mass spectral analysis supported the assigned structure. Anal. ($C_{14}H_{15}N_5O_7 \cdot 0.2H_2O$) C, H, N, H_2O.

2-Amino-7-propyl-9-(5′-phosphono-β-D-ribofuranosyl)-purine-6,8(1*H*)-dione 2.5Hydrate Dilithium Salt (57). To a solution of **6** (2.00 g, 5.89 mmol) and 4-(dimethylamino)-pyridine (0.72 g, 5.89 mmol) in dry pyridine (10 mL) was added diphenyl chlorophosphate (1.25 mL, 5.89 mmol). On addition of the diphenyl chlorophosphate most of the solids went into solution and the reaction mixture exothermed briefly. The solution was allowed to stir overnight under an argon atmosphere. MeOH was added (ca. 5 mL), and the solvents were then removed under vacuum. The product was purified on silica gel (CH_2Cl_2/MeOH/NH_4OH, 90:9:1) to give the 5′-bis-(phenylphosphoryl) ester of **6** (1.0 g, 30%). A fraction of this material (413 mg) was recrystallized from 95% EtOH to give 340 mg of analytically pure needles (205–207 °C): $[\alpha]^{25}_D$ 9.2° (*c* 0.76, MeOH); MS (FAB-thio) *m/e* 572 (MH^+). Anal. ($C_{25}H_{26}N_5O_9P \cdot 0.2H_2O$) C, H, N, H_2O. A solution of this material (0.47 g, 0.8 mmol) and PtO_2 (470 mg) in MeOH (50 mL) was shaken under 45 psig of hydrogen at room temperature overnight. The reaction was then adjusted to ca. pH 8 with aqueous LiOH, filtered through a Millipore filter, and then concentrated to give 0.24 g of a white glass. This sample was triturated with iPrOH, followed by ether, to give 0.21 g (60%) of **57** (mp >225 °C dec): ^1H NMR (D_2O) δ 0.89 (t, 3H), 1.71 (m, 2H), 3.9 (m, 3H), 4.08 (m, 1H), 4.2 (m, 1H), 4.54 (t, 1H), 5.16 (t, 1H), 5.81 (d, 1H). Anal. ($C_{13}H_{17}N_5O_9P \cdot 2Li \cdot 2.5H_2O$) C, H, N, H_2O.

1,2,3,9-Tetrahydro-6-methyl-1-(2-piperidinoethyl)-3-β-D-ribofuranosyl)-5*H*-imidazolo[4,5-*d*]triazolo[1,5-*a*]pyrimidine-9,12-dione (59). A mixture of 1-amino-7-(2-piperidinoethyl)-8-oxoguanosine (6.36 g, 14.9 mM), acetic anhydride (8 mL), triethylamine (13 mL), and CH_2Cl_2 (100 mL) was stirred at room temperature under nitrogen for 60 h and concentrated in vacuo. The residue was treated with concentrated ammonia (5 mL) and methanol (100 mL), and the mixture was stirred for 120 h. Most of solvent was removed in vacuo, and the residue was stirred in methanol (10 mL). The solid which formed was filtered, washed with cold water and acetone, and dried in a vacuum oven at 60 °C overnight to give **59** as an off-white powder (724 mg, 12%): mp >230 °C; IR (KBr) 1710, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.58 (d, *J* = 5 Hz, 1H, OH), 2.27 (s, 3H, CH_3). Anal. ($C_{19}H_{27}N_7O_6$) C, H, N.

2-Amino-7-(2-propenyl)-9-[1-(2,3-dideoxy-β-D-glycero-pent-2′-enofuranosyl)]purine-6,8(1*H*)-dione 0.3Hydrate (63). A suspension of **6** (5 g, 14.8 mmol) in CH_3CN (20 mL) was treated with 1-acetoxyisobutyryl bromide (15.5 g, 74 mmol). The mixture was heated at reflux for 30 min. The solution was cooled and treated with saturated aqueous $NaHCO_3$ and CH_2Cl_2. The CH_2Cl_2 layer was dried ($MgSO_4$), filtered, and concentrated. The residue was purified on a dry silica gel column (CH_2Cl_2/MeOH, 96:4) to give a rust-colored solid of the expected mixture of vicinal 2′,3′-bromoacetates (7.5 g, 90%), slightly unstable at room temperature. The majority of this material was dissolved in EtOH (80 mL) and treated with Zn dust (9 g) and HOAc (0.5 mL). As the conversion was slow at room temperature, after 4 h additional Zn dust (3 g) was added and the solution heated at 40 °C. After 40 h, the suspension was filtered through a course filter and then a Millipore filter and concentrated. This residue was then treated with toluene and evaporated, dissolved in MeOH (10 mL), and treated with 25% NaOMe/MeOH (10 mL). After stirring overnight, TLC showed the major formation of a lower spot. HOAc (ca. 4 mL) was added, and the product was extracted into $CHCl_3$ from water. The $CHCl_3$ layer was dried ($MgSO_4$), filtered, and concentrated. It was then purified on 200 g of silica (CH_2Cl_2/MeOH/ether, 94/6), from which 1.7 g nearly pure product was isolated. Recrystallization from CH_2Cl_2/ether followed by

recrystallization from MeOH/benzene gave 750 mg of **63**. The compound was dried under vacuum overnight at 60 °C which caused ca. 2% of a lower impurity spot to form by TLC determined by serial dilution of the the TLC sample, presumably due to thermal cleavage of the glycosidic bond: mp 122 °C, softening, 136–144 °C dec; $[\alpha]^{25}_D$ 74.1 (*c* 0.46, H_2O); IR (KBr) 3342, 1680, 1633, 1597, 1441 cm^{-1}; CI-MS (NH_3) *m/e* 306 (M + 1, 5), 208 (100); ^1H NMR (D_2O) δ 3.7 (br s, 2H), 4.33 (s, 2H), 4.8–4.9 (m, 2H), 5.02 (d, 1H), 5.8 (m, 1H), 5.91 (d, 1H), 6.17 (d, 1H), 6.62 (s, 1H), 7.24 (s, ca. 1H). The ^1H NMR indicated the presence of a small amount of benzene of recrystallization which was not removed during the drying. Anal. ($C_{13}H_{15}N_5O_4$·0.3PhH·0.3H_2O) C, H, N, H_2O.

2-Amino-7-propyl-9-[1-(2′,3′-dideoxy-β-D-glycerofuranosyl)]purine-6,8(1*H*)-dione Hemihydrate (64). A solution of **63** (370 mg) and 10% Pd/C (200 mg) in MeOH (10 mL) was stirred under an atmosphere of hydrogen. After 6 h, the sample was removed and filtered, and the charcoal pad was washed with additional MeOH. The filtrate was concentrated, and the residue was subjected to chromatography on silica gel (CH_2Cl_2/MeOH, 96:4). The major fraction was collected, recrystallized from MeOH/Et_2O, and then dried at 65 °C under vacuum overnight to give 180 mg of **64**: mp 138–140 °C; $[\alpha]^{25}_D$ −13.1° (*c* 0.45, H_2O); IR (KBr) 3350, 1678, 1631, 1594 cm^{-1}; CI-MS (NH_3) *m/e* 310 (M + 1, 45); 400-MHz ^1H NMR consistent with the structure: the H1′ signal comes at δ 5.92 (t, 1H). A trace amount of ether (ca. 0.1 molar equiv) was seen in the ^1H NMR spectrum. Anal. ($C_{13}H_{19}N_5O_4$·0.1$C_4H_{10}O$·0.5H_2O) C, H, N, H_2O.

2-(2,2-Dimethylpropanamido)-7-propyl-9-[1-(2′,3′-dideoxy-β-D-glycerofuranosyl)]purine-6,8(1*H*)-dione (65). A solution of **64** (1.0 g, 3.2 mmol), trimethylacetyl chloride (0.7 g, 6.5 mmol), and triethylamine (0.68 g, 6.7 mmol) in acetonitrile (40 mL) were heated to 50 °C overnight under an argon atmosphere. The solvent was evaporated under reduced pressure. The residue was dissolved in chloroform, washed with acid (1 N HCl), dried ($MgSO_4$), and filtered. The components were separated on four tapered prep plates. The major band was scraped off and extracted with methanol. Trituration of the millipore filtered methanol solution with ethyl ether followed by filtration and drying overnight in vacuo afforded 133 mg (11%) of a white powder of **65**: mp 102–103 °C; MS (CI-CH_4) *m/e* 394 (M + 1); ^1H NMR ($CDCl_3$) δ 0.9 (t, 3H), 1.35 (s, 9H), 1.8 (q, 2H), 2.1–2.4 (m, 3H), 2.5 (m, 1H), 3.6 (d, 1H), 4.0 (m, 3H), 4.3 (m, 1H), 5.2 (s, 1H), 6.1 (t, 1H), 7.3 (s, 1H), 8.2 (s, 1H), 12.0 (s, 1H). Anal. ($C_{18}H_{27}N_5O_5$) C, H, N.

2-Acetamido-7-propyl-9-[1-(5-acetyl-2′,3′-dideoxy-β-D-glycerofuranosyl)]purine-6,8(1*H*)-dione 0.2Hydrate (66). This compound was prepared in a similar manner as for the preparation of **65** using acetyl chloride, except that the diacylated product was obtained and purified as a tan oil in 50% yield. Anal. ($C_{17}H_{23}N_5O_6$·0.2H_2O).

2-(2-Methoxyacetamido)-7-propyl-9-[1-(2′,3′-dideoxy-5-(2-methoxyacetyl)-β-D-glycerofuranosyl)]purine-6,8(1*H*)-dione (67). The title compound was synthesized as for **65**, except that methoxyacetyl chloride was used as the acylating agent, and the product was obtained and purified in 53% yield: $[\alpha]^{25}_D$ −0.7° (*c* 1.0, MeOH). MS and NMR data were consistent with the structural assignment. Anal. ($C_{19}H_{27}N_5O_8$) C, H, N.

2-Amino-7-(2-propenyl)-9-[3′,5′-(tetraisopropyldisiloxanediyl)-β-D-ribofuranosyl]purine-6,8(1*H*)-dione (68). A solution of **6** (2.7 g, 8.0 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.2 mL, 9.7 mmol) in DMF (12 mL) and pyridine (4 mL) was stirred overnight under a drying tube. The mixture was then added to ice water, and the product was extracted into $CHCl_3$ (2×), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography on silica gel (400 g, CH_2Cl_2/MeOH, 95:5) to remove several close-running impurities to give 1.3 g (30%) of pure **68**: CI-MS (NH_3) *m/e* 582 (M + 1); 400-MHz ^1H NMR ($CDCl_3$) was consistent with the structure.

2-Amino-7-(2-propenyl)-9-[2′-[phenoxy(thiocarbonyl)]-3′,5′-(tetraisopropyldisiloxanediyl)-β-D-ribofuranosyl]-purine-6,8(1*H*)-dione (71). A solution of **68** (0.65 g, 1.37 mmol) in CH_3CN (10 mL) was treated with phenyl chlorothionocarbonate (180 μL) and 4-(dimethylamino)pyridine (280 mg). After stirring for 2 h, the solution was added to water, and the yellow solid which emerged was filtered and dried to give structure **71** (0.88 g).

2-Amino-7-(2-propenyl)-9-β-D-arabanosylpurine-6,8-(1*H*)-dione (70). A solution of **68** (0.89 g, 1.5 mmol) in DMSO (7 mL) was treated with Ac_2O (1 mL) and then allowed to stir overnight. This produced a 7:3 mixture of ketone **69** and the 2′-[(methylthio)methyl] ether byproduct.[26] As these two components were not readily separable, the solution was cooled to ca. 0 °C and then treated with $NaBH_4$ (2 × 150 mg). After warming to room temperature, water and EtOAc were added. The organic layer was withdrawn, dried ($MgSO_4$), filtered, and concentrated. This oil was purified on silica gel (CH_2Cl_2/MeOH, 95:5) to give a tacky semisolid which solidified further upon trituration with water (0.7 g). NMR and MS results confirmed the structure as being that of the TIPS protected derivative of **70**. A solution of this material (0.5 g, 0.84 mmol) was treated with 0.3 M HCl in MeOH/water (7:3, 20 mL) and allowed to stir for 3 d. EtOAc (ca. 0.5 L) was added, and the white precipitate which emerged was filtered and purified by semipreparative HPLC (reversed phase, MeOH/water, 1:1) and recrystallization from MeOH/Et_2O to give 100 mg of a mixture of **70**:**6** (92:8): mp 154 °C dec; IR 3340, 2927, 1677, 1634, 1598 cm^{-1}; $[\alpha]^{25}_D$ −30° (*c* 0.2, MeOH); CI-MS (NH_3) *m/e* 339 (M + 1); 400-MHz ^1H NMR (D_2O) was consistent with the structure: δ for H1′ arabino stereochemistry 6.08 (d, 0.92H, *J* = 7.2 Hz), δ for H1′ ribo stereochemistry 5.68 (d, 0.08 H, *J* = ca. 7 Hz). Anal. ($C_{13}H_{17}N_5O_6$) C, H, H_2O; N: calcd, 20.64; found, 20.00.

2-Amino-7-(2-propenyl)-9-(2′-deoxy-β-D-ribofuranosyl)-purine-6,8(1*H*)-dione 1.6Hydrate (73). The majority of **71** prepared above (0.75 g, 1.046 mmol) was dissolved into toluene (10 mL), and the solution was degassed with argon. AIBN (65 mg, 0.4 mmol) and tributyltin hydride (533 mL, 2 mmol) were added. The solution was heated at reflux for 20 h under nitrogen. The solution was concentrated and the product was purified on silica gel (500 g, CH_2Cl_2/MeOH, 96:4) to give 370 mg of **72** (60%): MS (FAB) *m/e* 586 (M + 1). This material was dissolved in MeOH (1.6 mL) and treated with 1 N HCl (0.4 mL). After stirring overnight, the solvent was removed and the product was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 8:2) and recrystallization from MeOH/Et_2O to give 80 mg of pure **73**: mp 166–174 °C dec; IR (KBr) 3420, 3220, 2921, 1681, 1629, 1594 cm^{-1}; CI-MS (NH_3) *m/e* 324 (M + 1, 40). The 400-MHz ^1H NMR spectrum was consistent with the structure. Anal. ($C_{13}H_{17}N_5O_5$·1.6H_2O): C, N; H: calcd, 5.78; found, 5.28; H_2O: calcd, 8.19; found, 2.19.

3,5′-Anhydro-7-methyl-8-oxoguanosine (75). To a mixture of **2** (5 g, 15.97 mM), triphenylphosphine (16 g, 61.07 mM), imidazole (4.20 g, 61.76 mM), and *N*-methylpyrrolidone (70 mL) was added iodine (15 g, 59.10 mM) in three portions at room temperature under nitrogen. The resulting solution was stirred for 16 h and then poured into a mixture of water (250 mL) and methylene chloride (600 mL) with stirring. The solid which formed was filtered and purified by preparative HPLC (C-18 reverse-phase column, MeOH/water, 1:9) to give **75** as a white powder (1.95 g, 41%): mp >230 °C; IR (KBr) 1703, 1650, 1628, 1600 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 6.93 (bs, 2H, exchangeable, NH_2), 5.71 (s, 1H, $C_{1'}$), 3.41 (s, 3H, CH_3). Anal. ($C_{11}H_{13}N_5O_5$) C, H, N.

5′-Deoxy-5′-iodo-7-methyl-8-oxoguanosine (74). Continued elution of the column described above with MeOH–water (2:8–3:7) provided the title compound as a white powder (1.52 g, 22%): mp 218 °C dec; IR (KBr) 1705, 1680, 1630, 1600 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.78 (bs, 1H, NH, exchangeable), 6.42 (bs, 2H, exchangeable, NH_2), 5.54 (d, *J* = 6 Hz, 1H, $C_{1'}$), 3.33 (AB q, 2H, CH_2I), 3.31 (s, 3H, CH_3N). Anal. ($C_{11}H_{14}IN_5O_5$) C, H, N.

5′-Deoxy-7-methyl-8-oxoguanosine Sesquihydrate (76). A mixture of **74** (1 g, 2.36 mM), tributyltin hydride (16 g, 61.07 mM), AIBN (4.20 g, 61.76 mM), and toluene (300 mL) was heated at 100 °C (bath temperature) under nitrogen for 2.5 h. The reaction was carefully monitored by analytical HPLC (C-18 reverse phase column, MeOH–water, 3:7). The resulting mixture was cooled to room temperature, and the solvent was

removed in vacuo. The residue was treated with ether (300 mL) with stirring. The solid was filtered and washed with ether. The solid was dissolved in water and purified by preparative HPLC (C-18 reverse-phase column, MeOH–water, 2:8–3:7) to give **76** as a white powder (405 mg, 47%): mp 195–198 °C; IR (KBr) 1703, 1650, 1628, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.74 (bs, ^{1}H, exchangeable, NH), 6.93 (bs, 2H, exchangeable, NH_2), 5.54 (d, 1H, J = 6 Hz, $C_{1'}$), 3.28 (s, 3H, CH_3N), 1.15 (d, J = 7 Hz, 3H, CH_3C). Anal. ($C_{11}H_{15}N_5O_5$·1.75H_2O) C, H, N.

2-Amino-7-(2-propenyl)-8-thioxo-9-β-D-glucopyranosylpurine-6(1*H*)-one 0.7Hydrate (81). A solution of 9-β-glucosylguanine[28] (3.5 g, 11.6 mmol) was dissolved in water (120 mL) and treated with bromine (0.90 mL, 17.4 mmol). After 1 h, the solution was cooled and the precipitated solid was collected. After drying there was 2.5 g of slightly tan powder of 8-bromo-9-β-glucosylguanine which was somewhat unstable upon heating: FAB-MS m/e 392 (M + 1, base peak). A portion of this solid (1.3 g, 3.32 mmol) and thiourea (1.14 g, 15 mmol) was dissolved in DMF (10 mL) and heated at 75 °C. After 3 h, the solution was cooled and treated with EtOAc (ca. 3 volumes) causing a red oil to emerge. The supernatant was decanted, the oil washed with additional EtOAc, dissolved in MeOH, and treated with 5 volumes of ether to produce a slightly yellow powder (1.1 g) of 8-thio-9-β-glucosylguanine: FAB-MS m/e 346 (M + 1, 40); ^{1}H NMR (DMSO-d_6) δ 3.18 (m, 1H), 3.3 (m, 2H), 3.4 (m, 1H), 3.72 (t, 1H), 5.78 (d, 1H), 6.60 (s, 2H, exchangeable). A portion of this solid (1.0 g) was dissolved in DMSO (4 mL) and treated with allyl bromide (400 μL, 4.73 mmol). After 30 min, ether was added causing an oil to emerge. The oil was washed twice more with ether and then treated with 20 mL of MeOH/water, 1:1. A small amount of 10% Na_2CO_3 was added to adjust the pH to 7. After refluxing overnight, no change had occurred, so the solvent was removed, and the residue redissolved in DMF (20 mL) and heated at 130–140 °C for 3 d. The solution was cooled and then treated with ca. 10 volumes of ether. The oil which resulted was purified by semipreparative reversed-phase HPLC (MeOH/water, 1:4) and then recrystallized from MeOH/ether. After drying at 70 °C overnight, 320 mg of pure **81** was obtained: mp >189 °C dec; IR (cm^{-1}) 3344, 2932, 1692, 1635, 1599, 1444, 1074; CI-MS (NH_3) m/e 386 (M + 1, 5), 264, 224 (M – Glu, 100); $[\alpha]^{25}_D$ +16.3° (c 1.00, water); ^{1}H NMR (DMSO-d_6, D_2O added) δ 3.7 (m, 3H), 3.88 (dd, 2H), 4.8–5.0 (m, 4H), 5.22 (d, 1H), 6.0 (m, 1H), 6.19 (d, 1H). Anal. ($C_{14}H_{19}N_5O_6S$·0.7H_2O) C, H, N, S, H_2O.

2-Amino-7-(2-propenyl)-9-[1(*R*)-[[bis(hydroxymethyl)-methyl]oxy]-2-hydroxyethyl]purine-6,8(1*H*)-dione Hydrate (83). To a mixture of **7** (2.5 g, 7.1 mM) and water (100 mL) was added a solution of sodium periodate (1.51 g, 7.1 mM) at room temperature under nitrogen. After stirring for 6 h, the resulting solution was cooled to 0 °C, and an excess of sodium borohydride (3.1 g, 81 mM) was added in three portions. After addition, the mixture was allowed to warm to room temperature and stir overnight. The clear solution was purified by preparative HPLC (C-18 reverse-phase column, MeOH/water, 1:9–2:8) to give **83** (1.75 g, 70%) as a white powder: mp 162–165 dec; IR (KBr) 1710, 1680, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 11.1 (bs, 1H, exchangeable, NH), 6.85 (bs, 2H, exchangeable, NH_2), 6.60 (s, 1H, $C_{1'}$), 2.01 (s, 3H, CH_3CO), 1.45 and 1.32 (both s, 3H each). Anal. ($C_{13}H_{19}N_5O_6$·H_2O) C, H, N; H: calcd, 5.89; found, 5.47.

2-Amino-7-(2-propenyl)-9-[2(*R*)-[4-butyl-6(*S*)-(hydroxymethyl)morpholino]]purine-6,8(1*H*)-dione 0.4Hydrate (84). A suspension of **6** (2 g) in water (40 mL) was treated with $NaIO_4$ (1.52 g, 1.2 molar equiv) with cooling in an ice/water bath. The suspended sample of **6** went into solution over the period of several minutes. After 15 min, $BuNH_2$ was added (0.58 mL, 1 molar equiv) followed by immediate addition of HOAc (ca. 0.2 mL) to adjust the pH in the range of 6–7. $NaCNBH_3$ (0.72 g) was added, resulting in considerable bubbling (the pH was still in the 6–7 range). After 1 h, $CHCl_3$ was added to the solution along with saturated aqueous $NaHCO_3$. After extraction into $CHCl_3$ (3×), the organic layers were combined, dried ($MgSO_4$), filtered, and concentrated. The residue was purified on silica gel (CH_2Cl_2/MeOH, 92:8) and by recrystallization (MeOH/Et_2O/pentane) to give **84** as a white solid which was dried at 70 °C overnight under vacuum (170 mg, 15%): mp 135–150 °C dec; IR (KBr) 3300–3500, 2940, 1688, 1640, 1600 cm^{-1}; $[\alpha]^{25}_D$ –18.6° (c 1.00, MeOH); MS (FAB) m/e 379 (M + 1, 20); 400-MHz ^{1}H NMR ($CDCl_3$; key morpholine resonances only) δ 2.18 (t, 1H, H2′-ax), 2.72 (t, 1H, H2′-eq or H4′-eq), 2.80 (t, 1H, H2′-eq or H4′-eq), 3.16 (t, 1H, H4′-ax), 5.68 (AB q, 1H, H1′, J = 1,9 Hz). Anal. ($C_{17}H_{26}N_6O_4$·0.4H_2O) C, H, N, H_2O.

2-Amino-7-(2-propenyl)-9-[2(*R*)-[4-(diphenylmethyl)-6(*S*)-(hydroxymethyl)morpholino]]purine-6,8(1*H*)-dione 0.2Hydrate (85). This compound was prepared in a similar manner as that described for the preparation of **84**, to give **85** (15%), recrystallized from MeOH/water (mp 273–275 °C); $[\alpha]^{25}_D$ –19.1° (c 0.47, MeOH/$CHCl_3$, 2:1). 400-MHz ^{1}H NMR (DMSO-d_6 was the best solvent for sharp resolution) and MS data support the structure. Anal. ($C_{26}H_{28}N_6O_4$·0.2H_2O) C, N, H_2O; H: calcd, 5.82; found, 5.30.

10*H*,12*H*,13*H*-1,5,3-Dioxazepino[2,3-*e*]-9H-purin-6(1*H*)-one (88). 8-Bromoacyclovir (**87**, 340 mg) was dissolved into DMSO (1.8 mL) and treated with 80% NaH/oil dispersion (80 mg). The solution was heated at 65 °C for 4 h, whereupon TLC showed a ca. 60% conversion to a less polar spot. An additional 30 mg of the 80% NaH/oil dispersion was added, the solution was heated an additional 30 min, and the solution was than cooled. The product was purified by semipreparative HPLC (reversed phase, MeOH/water, 15:85) and then recrystallized from water to afford **88** as a white solid (130 mg): mp >310 °C; IR (KBr) 3384, 3311, 3144, 1689, 1584 cm^{-1}; CI-MS (NH_3) m/e 224 (M + 1); 400-MHz ^{1}H NMR was consistent with the structure. Anal. ($C_8H_9N_5O_3$) C, H, N.

2-Amino-9-[(2-hydroxyethoxy)methyl]-7-(2-propenyl)-8-thioxo-9*H*-purin-6(1*H*)-one 0.2Hydrate (90). 8-Bromoacyclovir (**87**, 2.8 g) was suspended into DMF (12 mL) and treated with thiourea (3.5 g), and the mixture was heated at 80 °C for 6 h. The solution was cooled and then treated with an equal volume of water, and the solid precipitate of 8-thioacyclovir (**89**, 3.1 g) was collected: CI-MS m/e 258 (M + 1, base peak). The majority of this solid (3.0 g) was dissolved in DMF (15 mL) and treated with allyl bromide (3.5 mL). The solution was heated briefly (30 min) at 70 °C, whereupon TLC showed a very clean conversion to an upper spot. The solution was cooled and then treated with 3 volumes of water. The flocculent white solid which emerged was collected and dried to give 2.0 g of 8-(allylthio)acyclovir. A portion of this material (1 g) was recrystallized from boiling water to give an analytically pure sample: mp 216–216 °C; FAB-MS m/e 298 (M + 1, base peak); ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 4H), 3.80 (d, 2H), 4.67 (t, 1H, exchangeable), 5.09 (d, 1H), 5.20 (d, 1H), 5.28 (s, 2H), 5.91 (m, 1H), 6.55 (s, 1H, exchangeable), 11.0 (s, 1H, exchangeable). Anal. ($C_{11}H_{15}N_5O_3S$) C, H, N, S, H_2O. The other fraction of 8-(allylthio)acyclovir that was not recrystallized (1 g) was dissolved in DMF (20 mL) and heated at 130–140 °C for 3 d. The solution was cooled and then treated with ether. The supernatant was decanted, and the residual oil was triturated in water to cause precipitation of a white solid which was recrystallized from MeOH/ether. The first crop which emerged was pure by TLC; this sample was dried under vacuum for 1 d at 75 °C to give 360 mg of **90** (mp 215–217 °C): FAB-MS m/e 298 (M + 1, base peak); ^{1}H NMR (DMSO-d_6) δ 3.43 (m, 2H), 3.63 (t, 2H), 4.61 (t, 1H, exchangeable), 4.89 (d, 2H, exchangeable), 5.03 (d, 1H), 5.15 (d, 1H), 5.46 (s, 2H), 5.92 (m, 1H), 6.78 (s, 2H, exchangeable), 11.08 (s, 1H, exchangeable). Anal. ($C_{11}H_{15}N_5O_3S$·0.2H_2O) C, H, N, S, H_2O.

***N*^{6}-(4-Hydroxybutyl)-2,5,6-triamino-4-chloropyrimidine (93, R = 1-Hydroxybutyl).** A mixture of 2-amino-4,6-dichloropyrimidine (20 g, 122 mM), 4-aminobutanol (10.7 g, 120 mM), K_2CO_3 (10 g), and ethanol (500 mL) was heated to reflux under nitrogen for 18 h. The resulting mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo, and the residue was triturated with ethyl acetate. The solid was filtered and dried in a vacuum oven to give N^6-(4-hydroxybutyl)-2,6-diamino-4-chloropyrimidine (22.4 g, 84%) as a yellow solid, mp 110–112 °C. To a mixture of this material (18.0 g, 83 mM), sodium acetate (88 g), acetic acid (400 mL), and water (400 mL) was added a

solution of 4-chlorobenzenediazonium chloride [prepared from 4-chloroaniline (10.6 g) and sodium nitrite (6 g) in 3 N HCl (90 mL) according to the literature procedure[31]] dropwise at room temperature under nitrogen. After 18 h of stirring, the resulting mixture was filtered. The solid was washed with water and then air-dried. To a mixture of this crude (4-chlorophenyl)azo derivative in ethanol (400 mL), water (400 mL), and acetic acid (60 mL) was added zinc dust (60 g) in portions at 60 °C under nitrogen and stirred for 4 h. The resulting mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo, and the residue was treated with water (400 mL). This was extracted with ether (2 × 100 mL) and the aqueous layer was concentrated in vacuo to give a brown tar. This was treated with ethanol (500 mL) with stirring and the solid was filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on Florisil (800 g, CH_3OH/EtOAc, 5:95) to give **93** (R = 1-hydroxybutyl) as beige crystals (75%): mp 110–111 °C; IR (KBr) 1703, 1650, 1628, 1600 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.40 (t, J = 6 Hz, 1H, exchangeable, NH), 5.50 (bs, 2H, exchangeable, 2-NH_2), 4.30 (t, J = 6.5 Hz, 1H, OH), 3.80 (bs, 2H, 5-NH_2), 3.35 (m, 4H, CH_2NH and CH_2OH), 1.50 (m, 4H, CH_2CH_2). Anal. ($C_8H_{14}ClN_5O$) C, H, N.

2-Amino-6-chloro-9-(4-hydroxybutyl)-8-oxo-7*H*-purine Hemihydrate (94, R = 1-Hydroxybutyl). To a mixture of **93** (R = 1-hydroxybutyl, 2.0 g, 8.7 mM), triethylamine (2.5 mL, 18 mM), and THF (50 mL) was added a solution of phosgene in toluene (6.8 mL, 1.93 M, 13 mM) dropwise at 0 °C under nitrogen during a 30 min period. After addition, the mixture was stirred for an additional h and water (1 mL) was added. The resulting mixture was filtered and washed with ethyl acetate and cold water to give a light brown solid. This was recrystallized from methanol to afford **94** (R = 1-hydroxybutyl) as an off-white powder (1.5 g): mp 222–225 °C; IR (KBr) 3420, 1710, 1640, 1590 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.20 (bs, 1H, exchangeable, NH), 6.50 (bs, 2H, exchangeable, 2-NH_2), 4.35 (t, J = 6.5 Hz, 1H, OH), 3.70 (t, J = 6.5 Hz, 2H, NCH_2), 3.35 (m, 2H, CH_2OH), 1.50 (m, 4H, CH_2CH_2). Anal. ($C_9H_{12}ClN_5O_2 \cdot 0.5H_2O$) C, H, N.

7-Allyl-2-amino-6-chloro-9-(4-hydroxybutyl)-8-oxo-7*H*-purine Hemihydrate (95, R = 1-Hydroxybutyl). To a solution of **94** (R = 1-hydroxybutyl, 0.8 g, 3.1 mM) in DMF (15 mL) was added NaH (0.12 g, 60% oil dispersion, 3 mM) all at once at 0 °C under nitrogen, and the mixture was stirred for 30 min. To this was added allyl bromide (0.37 g, 3.1 mM), and the resulting mixture was allowed to warm to room temperature and stirred overnight. Most of solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (120 g, CH_2Cl_2/CH_3OH/concentrated NH_4OH, 100/5/0.1) to give **95** (R = 1-hydroxybutyl) as an off-white powder (40%): mp 132–133 °C; IR (KBr) 3320, 1715, 1640, 1585, 1460 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.70 (bs, 2H, exchangeable, NH_2), 5.95 (m, 1H), 3.70 (t, J = 6.5 Hz, 2H, NCH_2), 3.35 (m, 2H, CH_2OH), 1.50 (m, 4H, CH_2CH_2). Anal. ($C_{12}H_{16}ClN_5O_2 \cdot 0.5H_2O$) C, H, N.

7-Allyl-2-amino-9-(4-hydroxybutyl)purine-6,8(1*H*)-dione (98). A mixture of **95** (R = 1-hydroxybutyl, 0.45 g, 1.5 mM) and 0.5 N NaOH (30 mL) was heated to reflux under nitrogen for 6 h. The resulting mixture was cooled to room temperature and extracted with CH_2Cl_2 (3 × 20 mL). The aqueous phase was acidified to pH 6 with dilute HCl, and most of the solvent was removed under vacuum. The solid residue was recrystallized from ethanol to give **98** as a white powder (0.26 g, 54%): mp 207–211 °C; IR (KBr) 3320, 1715, 1640, 1585, 1460 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.65 (bs, 1H, exchangeable, NH), 6.45 (bs, 2H, exchangeable, NH_2), 5.95 (m, 1H), 3.60 (t, J = 6.5 Hz, 2H, NCH_2), 3.35 (m, 2H, CH_2OH), 1.50 (m, 4H, CH_2CH_2). Anal. ($C_{12}H_{17}N_5O_3$) C, H, N.

Murine Adjuvanticity. Viable CBA/CaJ mouse B cells were cultured by treating 10^8 spleen cells with a 1:1000 dilution of monoclonal anti-T cell antibody for 30 min at 4 °C. Treated cells were centrifuged, and the cells were resuspended in a 1:6 dilution on CBA RBC-absorbed guinea pig complement at 37 °C for 45 min. These cells were then incubated with or without 0.1 mL of 0.1% (v/v) SRBC as antigen in serum-containing media further containing incremental amounts of a guanosine derivative ranging in amount from zero to 10^{-3} M. After 4 d, the cells were plaqued in standard low MW agarose (Bio-Rad Laboratories, Richmond, CA) and were incubated in SRBC-absorbed guinea pig complement for 1 h after a 1.5 h incubation without complement. After this period, the plaques were manually counted and evaluated.

Murine Mitogenicity. Compounds were dissolved in water or DMSO and diluted to the appropriate concentration with medium. The assay entailed tritiated thymidine incorporation by incubating murine spleen cells (CBA/CaJ mice; 1 × 10^5/0.2 mL) with various concentrations of the test compounds for 24 h in microculture plates. [^{3}H]Thymidine was then added to each well, and the incubation was further conducted for another 24 h. The cells were harvested, and the tritium labeled thymidine uptake was evaluated using a Beckman scintillation counter.

Natural Killer Cell Assay. The NK-sensitive cell line Yac-1, obtained from Dr. John Roder, Mount Sinai Hospital, Toronto, Ontario, was maintained in RPMI 1640 medium with 5% fetal calf serum (Hyolme Laboraties, Logan, UT) at 37 °C, 5% CO_2. Cell lines were tested periodically for mycoplasma and found to be negative. Male C3H/HcT mice, obtained from Jackson Laboratories, Bar Harbor, ME, were used between 8 and 12 weeks of age. They were fed Purina rodent laboratory chow and water ad libitum. Spleen cells obtained from the mice were cultured at concentrations of 5 × 10^6, 2.5 × 10^6, 1.25 × 10^6, and 6.2 × 10^5 with the test compounds, each in triplicate. The test compounds were added to the culture wells in a volume of 100 μL to give final concentrations of 3, 10, 30, 100, and 300 μM of drug. Cultures were incubated for 18 h at 37 °C in 5% CO_2. Yac-1 target cells (5 × 10^5) were incubated with 100 μCi $Na^{51}Cr$ (Amersham Canada, Oakville, Ontario) for 1 h at 37 °C, in 5% CO_2, washed three times with phosphate-buffered saline, and suspended in culture medium at 5 × 10^4/mL. The plates containing cells to be tested for cytotoxic activity were centrifuged at 220*g* for 10 min, 100 μL of supernatant was removed from each well, and 100 μL of labeled, Yac-1 target cells were added to the wells. The plates were centrifuged for 5 min at 150*g*, incubated at 37 °C in 5% CO_2 for 4 h, and centrifuged for 5 min at 300*g*. Supernatants were removed from the wells using the Skatron Supernatant Collection System (Skatron, Sterling, VA), and the samples were counted in a Beckman γ counter. The percent specific lysis was calculated for each well and lytic units (LU) of activity were calculated for each drug concentration.[34] The ED_{50} was calculated for each drug. The percent standard response was calculated as the [maximum LU-drug/maximum LU-**6**] × 100.

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