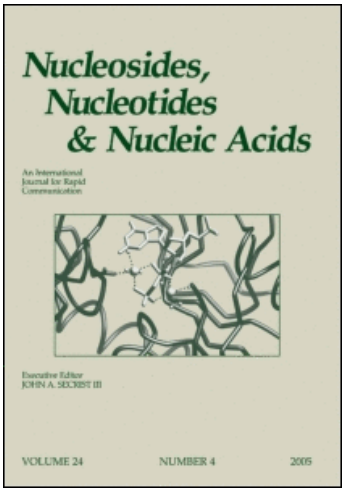


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Guanosine Derivatives as Immunostimulants. Discovery of Loxoribine

Robert Chen[ab]; Michael G. Goodman[ac]; Dennis Argentieri[ad]; Stanley C. Bell[ae]; Levelle E. Burr[ab]; Jon Come[ab]; Jacquelyn H. Goodman[ac]; Dieter H. Klaubert[ab]; Bruce E. Maryanoff[ae]; Barbara L. Pope[af]; Marianne S. Rampulla[ab]; Mary R. Schott[ae]; Allen B. Reitz[ae]
[a] The R. W. Johnson Pharmaceutical Research Institute (PRI) and The Scripps Research Institute (TSRI), [b] Medicinal Chemistry Department, PRI, Raritan, New Jersey, USA [c] Department of Immunology, TSRI, La Jolla, California, USA [d] Immunopharmacology, PRI, Raritan, New Jersey, USA [e] Medicinal Chemistry Department, PRI, Spring House, Pennsylvania, USA [f] Immunopharmacology, PRI, Ontario, Canada

To cite this Article Chen, Robert , Goodman, Michael G. , Argentieri, Dennis , Bell, Stanley C. , Burr, Levelle E. , Come, Jon , Goodman, Jacquelyn H. , Klaubert, Dieter H. , Maryanoff, Bruce E. , Pope, Barbara L. , Rampulla, Marianne S. , Schott, Mary R. and Reitz, Allen B.(1994) 'Guanosine Derivatives as Immunostimulants. Discovery of Loxoribine', Nucleosides, Nucleotides and Nucleic Acids, 13: 1, 551 — 562
To link to this Article: DOI: 10.1080/15257779408013262
URL: http://dx.doi.org/10.1080/15257779408013262

NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 551-562 (1994)

GUANOSINE DERIVATIVES AS IMMUNOSTIMULANTS. DISCOVERY OF LOXORIBINE

Robert Chen*,† Michael G. Goodman,‖ Dennis Argentieri,¢ Stanley C. Bell,‡ Levelle E. Burr,† Jon Come,† Jacquelyn H. Goodman,‖ Dieter H. Klaubert,† Bruce E. Maryanoff,‡ Barbara L. Pope,£ Marianne S. Rampulla,† Mary R. Schott,‡ and Allen B. Reitz*,‡

The R. W. Johnson Pharmaceutical Research Institute (PRI) and
The Scripps Research Institute (TSRI)

Abstract: 7-Allyl-8-oxoguanosine (loxoribine, **5**) was selected from a series of guanosine derivatives for further evaluation as an immunostimulant. Numerous related analogs were also synthesized and evaluated: 2',3'-ketals of **5** are particularly interesting because they are active, apparently without being cleaved to the free nucleoside.

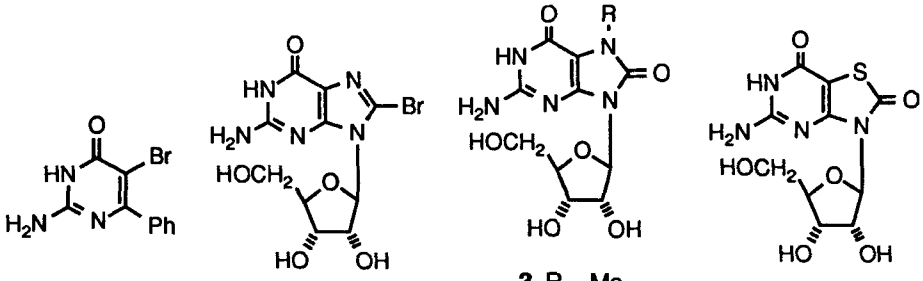
Drug therapy which stimulates the immune system offers an important means of treating viral infections or cancer.[1] Preparations which have been used clinically include oligosaccharide mixtures obtained from microbial sources (e.g. those related to Freund's adjuvant) and thymus-derived peptide extracts (e.g. thymomodulin).[2] In addition, there has been considerable research aimed at finding small-molecule (MW < 600) immunostimulants which could be developed as more traditional drug products. For example, bropirimine (**1**), a 2-pyrimidinone derivative, has been evaluated extensively as an immunostimulant in various clinical trials.[3]

Guanosine derivatives hold considerable promise as small-molecule immunostimulants.[1] 8-Bromoguanosine was initially prepared in 1964 by the Robins group;[4] its immunostimulatory properties were first described in 1981 by Goodman and Weigle,[5] leading to the evaluation of other 8-substituted guanosines and related structures.

This paper is submitted in honor of Dr. Roland K. Robins.

† PRI, Medicinal Chemistry Department, Raritan, New Jersey 08869 USA.
‖ TSRI, Department of Immunology, La Jolla, California 92037 USA.
¢ PRI, Immunopharmacology, Raritan, New Jersey 08869 USA.
‡ PRI, Medicinal Chemistry Department, Spring House, Pennsylvania 19477 USA.
£ PRI, Immunopharmacology, 19 Green Belt Dr., Don Mills, Ontario, Canada M3C 1L9.

7-Methyl-8-oxoguanosine (**3**),[6] and 7-thia-8-oxoguanosine (**4**)[7] are potent immunostimulants that have been described in detail by Goodman and Hennon, and by Robins and co-workers, respectively. These compounds are intracellular mitogens for B lymphocytes, and in the presence of antigen, stimulate the differentiation of B cells to a state of antibody secretion (in either the absence or presence of T helper cells),[8] a measure of adjuvanticity.



Our own research in this area emerged from an ongoing Johnson & Johnson-Scripps research collaboration. We have prepared over 80 diverse compounds, using compound **3** as our lead structure.[9] During the course of our work, we synthesized 7-allyl-8-oxoguanosine (loxoribine, **5**), which we are currently evaluating as an immunostimulant in humans. This paper describes the selection of loxoribine from several closely-related analogs, and the preparation and activity of several derivatives of **5** in which the 2',3'-hydroxyls are joined together in a ring.

Chemical Synthesis and Biological Testing. Since **3**, bearing a methyl group on N-7, is considerably more active than parent structure 8-oxoguanosine,[6] manipulation of the N-7 substituent was the starting point for our research. N^1-Aminoguanosine derivative **6** was synthesized and alkylated with a variety of electrophiles,[6b] followed by deprotection with sodium nitrite and HCl. We discovered early on that optimal activity was obtained when there was a medium-length alkyl chain (2-4 carbons) on N-7. Some of the simpler compounds prepared by this method are listed in Table 1, along with their biological activity in an assay measuring the increase in the antibody production after a sheep red blood cell (SRBC) antigen challenge.[9] All of these analogs were prepared by the method described above, except that propyl derivative **8** was synthesized from **5** by catalytic hydrogenation (H_2, Pd/C). The pronounced dependence of activity upon chain-length for the data shown in Table 1 is graphically displayed in Figure 1, where it can be clearly seen that a three carbon chain produced the most potent immunostimulatory response. Because of these and other data, **5** was chosen for further evaluation.[10] We have developed an

Table 1. Activity of 7-alkyl-8-oxoguanosines

Compound	R	Direct anti-SRBC PFC/Culture* (1×10^{-5} M)
3	Me	145
7	Et	270
8	Pr	1306
5	allyl	1387
9	Bu	900
10	2-(*E*)-butenyl	612
11	n-C_6H_{13}	243

* Adjuvanticity against SRBC measured in direct plaque-forming cells per culture of lymphocytes from mouse strains CBA/CaJ or C57BL/6J

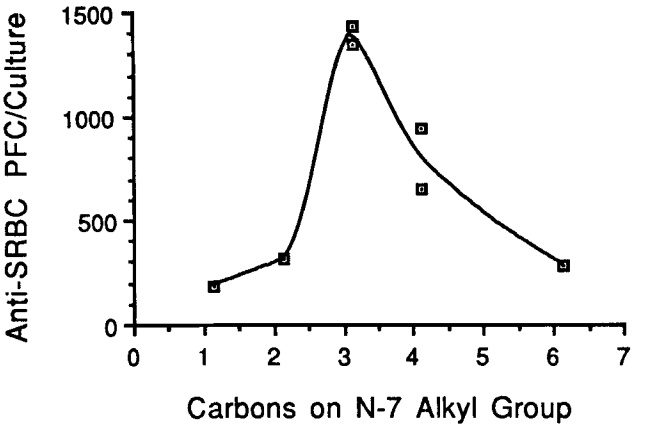
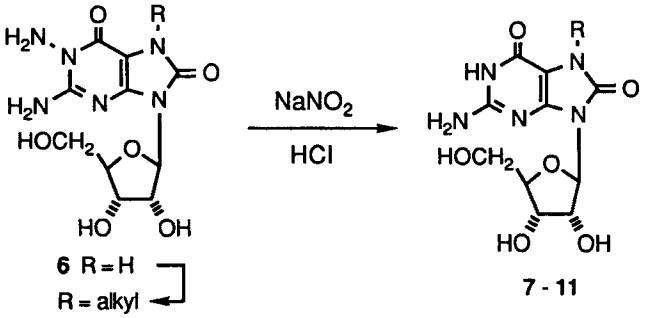
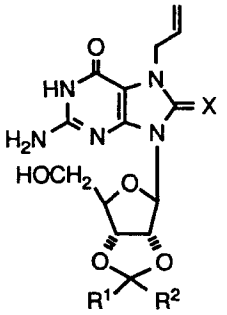


Figure 1. Dependence of Adjuvanticity Upon Chain Length for 7-Alkyl-8-oxoguanosines.

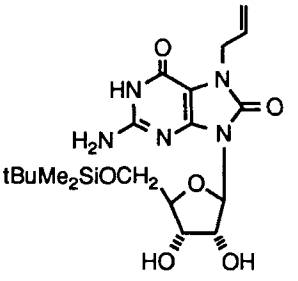
improved synthetic route for the preparation of **5** involving a Claisen-type rearrangement of 8-allyloxyguanosine,[11] which was amenable to the preparation of the large amounts required for clinical trials.



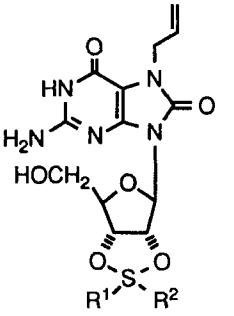
In order to carry out further synthetic manipulations on **5**, we blocked the 2' and 3' hydroxyls as an isopropylidene ketal (viz. **12**). Surprisingly, this compound and related structures displayed considerable biological activity on their own, prompting us to prepare a series of related structural variants, some of which we describe here (**12-14**). These ketals are probably not prodrugs by virtue of ketal cleavage, as this ketal functionality was determined to be stable to dilute acid (pH 2.0, 2 h, 24°C), and there does not appear to be an enzyme that would cleave this group. Compound **12** was prepared directly by reaction of the 8-thiono analog of **5**[11] with acetone, using H_2SO_4 or zinc chloride as catalyst. It was considerably more difficult to prepare ketals **13-14**, primarily due to the insolubility of **5**.



12 $R^1 = R^2 = Me$, X = S
13 $R^1, R^2 = (CH_2)_5$, X = O
14 $R^1, R^2 = (CH_2)_2O(CH_2)_2$, X = O



15



16 R^1 = O, R^2 = lone pair
17 $R^1 = R^2$ = O

The best conditions were those of Seela and Waldek,[12] using HCl/dioxane and triethyl orthoformate, which allowed for the preparation of ketals **13-14** from **5** and the appropriate ketones.

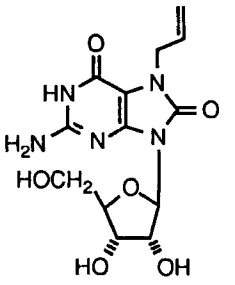
Compound **15** proved to be a useful intermediate for the selective functionalization of the 2',3'-hydroxyls of **5**. Sulfite **16** (3.4:1 mixture of diastereomers) was synthesized by reaction of $SOCl_2$ with **15**, followed by fluoride deprotection. Lastly, sulfate **17** was obtained by treatment of **15** with sulfuryl diimidazole,[13] followed by removal of the 5'-silyl protecting group under acidic conditions (p-TsOH, MeOH).

Compound **12** displayed ca. 20% of the adjuvanticity potency (dose at which 50% of the maximal response occurred) of **5**, while retaining 89% of the maximal activity (greatest activity at any dose, efficacy). Surprisingly, cyclohexylidene congener **13** was nearly equipotent to **5** in potency and efficacy, whereas inserting an oxygen into the cyclohexyl ring (**14**) resulted in a loss of ca. 90% of the activity. This indicates a requirement for a hydrophobic interaction for the adjuvanticity activity, which is disrupted in the presence of the oxygen atom. Sulfite **16** was similar in potency to **5**, although approaching only ca.

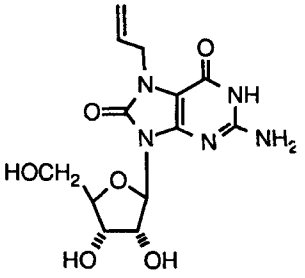
45% of the maximal response. Separate experiments demonstrated that **16** was stable under the conditions of the biological assay. Alternatively, sulfate **17** was essentially inactive in the adjuvanticity assay.

Physical Properties of **5**. Guanosine and its derivatives display only sparing solubility in water and organic solvents, and this is also true of many of the compounds we examined (0.9% aqueous solubility at 25°C). It is much more soluble in DMSO, and 1 N NaOH (228 mg/mL at 25°C). The solubility in aqueous base is presumably due to deprotonation of the heterocyclic ring (pKa 8.92). The octanol/water partition coefficient for loxoribine (log P) was determined to be a hydrophilic -2.00, indicating that it would not be expected to appreciably cross the blood-brain barrier.[14]

Conformational Analysis. Nucleosides adopt a variety of low-energy conformations as a consequence of rotation about the glycosidic C-N bond.[15] 8-Substituted guanosines are known to prefer a syn conformation in which the ribose ring is situated underneath the purine ring. This has been demonstrated in the solid state by crystal structure determinations, such as for **2**.[16] Alternatively, unsubstituted purine nucleosides display a preference for the anti conformation, because of the relief of steric repulsive interactions in the "syn" state. It is possible to correlate the observed ^{13}C NMR chemical shift for C-2', and the propensity of a nucleoside derivative to adopt the syn conformation, because of a characteristic ca. 3 ppm downfield shift of the syn-C2 resonance relative to that of anti-C2'.[17] Comparison of the C2' resonances for guanosine (δ 34.18 upfield relative to DMSO-d_6), **2** (30.86), 8-oxoguanosine (31.34) and loxoribine (31.28) reveals that loxoribine also displays this shift, and would be expected to reside mainly in the syn conformation in solution (X-ray structure not determined). It has been proposed that the immunostimulatory activity of **2** and related compounds is partly due to this syn conformation,[18] and the activity of loxoribine is consistent with this trend.



5 (loxoribine) - Syn



5 (loxoribine) - Anti

Conclusions. Based on its activity in the adjuvanticity assay described above, and in a variety of other in vitro and in vivo models, loxoribine (**5**) has been chosen for further

evaluation. It was selected after evaluating a large number of analogs, including closely-related 7-alkyl derivatives. Surprisingly, 2',3'-ketals of **5** have been prepared and display significant activity on their own, which diminishes in the presence of an electron-rich atom (e.g. oxygen) in the attached ketal. A sulfite analog was prepared (**16**) which was considerably active.

EXPERIMENTAL SECTION

General Procedures. ^{1}H NMR spectra were recorded on either a Bruker AM-360WB (360 MHz), Bruker AM-400 (400 MHz), or Varian 390 (90 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; only representative peaks are listed in the experimentals. Elemental analyses were mainly conducted by the Analytical Services group at PRI, Raritan, New Jersey; those samples requiring water analysis were evaluated by Robertson Microlit, Madison, New Jersey. Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are corrected. Chemical-ionization mass spectra (CI-MS) were recorded on a Finnigan 3300-6100 system with methane as the reagent gas.

General Synthesis of 7-Alkyl-2-amino-9-(β-D-ribofuranosyl)purine-6,8(1H)-diones (5, 7-11). To a solution of **6** (9.5 g, 30 mmol) in DMF (250 mL) was added NaOMe (33 mmol). After stirring at ambient temperature for 30 min, a solution of the alkylating agent (33 mmol) in DMF (10 mL) was added and the resulting solution was stirred for 16 h. The solvent was then removed and the solid that resulted was washed with water (150 mL) and CH_2Cl_2 (150 mL), and recrystallized from EtOH/water. The resultant material was then dissolved in concentrated HCl (e.g., 4.65 mmol in 15 mL of HCl), followed by addition of aqueous sodium nitrite (e.g. 4.19 mmol in 5 mL of water) at 0°C. The resulting mixture was neutralized with aqueous sodium acetate and then filtered. The filtrate was purified by preparative reversed-phase HPLC (MeOH/water). The fractions of the desired deaminated product were combined and the solvent was removed under vacuum. The resulting white solid was recrystallized from EtOH/water to give pure product, as a white powder. The yields of the two-step procedure were often low (10%), but some product was probably lost during the rigorous HPLC purification.

2-Amino-7-ethyl-9-(β-D-ribofuranosyl)purine-6,8(1H)-dione Hemihydrate (7). The general procedure was followed with ethyl iodide as the alkylating agent to afford **7** (15% yield), mp 185-187°C. ^{1}H NMR (90 MHz) δ 10.80 (bs, 1H, exchangeable, CONH), 6.7 (bs, 2H, exchangeable, NH_2), 5.7 (d, J=5 Hz, 1H, C_1'), 1.2 (t, J=6 Hz, 3H, CH_3) Anal. Calcd. for $C_{12}H_{17}N_5O_6 \cdot 0.5H_2O$: C, 42.86, H, 5.39; N, 20.82. Found: C, 43.17; H, 5.24; N, 20.45.

2-Amino-7-(2-propenyl)-9-(β-D-ribofuranosyl)purine-6,8(1H)-dione (5). (a) Via the two-step general method. The general procedure was followed with allyl bromide as the alkylating agent to afford **5** (40% yield), mp 234-236°C. IR (KBr) 1690, 1635, 1597, cm^{-1}. CI-MS m/e 340 (M+1). ^1H NMR (300 MHz) δ 10.88 (bs, 1H, exchangeable, NH), 6.51 (bs, 2H, exchangeable, NH_2), 5.91 (ddt, 1H, J=5.3, 10.3, 17.1 Hz), 5.62 (d, 1H, J=6 Hz), 5.27 (d, 1H, J=6.0 Hz), 5.12 (dd, 1H, J=1.4, 10.3 Hz), 5.05 (dd, 1H, J=1.5, 17.2 Hz), 4.96 (d, 1H, J=4.9 Hz), 4.85 (m, 1H), 4.78 (dd, 1H, J=4.9, 7.1 Hz), 4.41 (d, 2H, J=5.32 Hz), 4.10 (m, 1H), 3.80 (m, 1H), 3.56-3.63 (m, 1H), 3.41-3.49 (m, 1H). ^{13}C NMR (selected resonances in DMSO-d_6, 100 MHz) δ 30.38 (measured downfield from the highest peak of the DMSO-d_6 signal, C-3'), 31.28 (C-2'). Anal. Calcd. for $C_{13}H_{17}N_3O_6$: C, 46.02, H, 5.05; N, 20.64. Found: C, 45.63; H, 5.10; N, 20.56.

(b) Via a Claisen-type rearrangement. To a suspension of sodium hydride (60% oil dispersion, 5 g, 125 mM) in DMSO (200 mL) was added allyl alcohol (20 mL, 294 mM) dropwise at room temperature under nitrogen. To this was added 8-bromoguanosine (10 g, 29.5 mM) all at once and the resulting mixture was heated at 60°C (bath temperature) for 3 h. The mixture was allowed to cool to room temperature and then slowly poured into ether (1L). The ethereal layer was decanted and discarded. The solid residue was treated with water (50 mL) and acetic acid (10 mL). The resulting mixture was purified by preparative HPLC (C-18 reverse phase column, MeOH/water, 1:9) to give 2-amino-8-(2-propenyloxy)-9-[β-D-ribofuranosyl]purine-6-one as a white powder (1.8 g, 20%). On a larger scale (1.0 mole), process improvements were developed so that the chemical yield was improved to 62%. This material was about 95% pure by HPLC analysis and was used directly in the next step without further purification. A mixture of 2-amino-8-(2-propenyloxy)-9-[β-D-ribofuranosyl]purine-6-one (4.0 g), prepared as described above, in methanol/water (1:1, 50 mL) was heated to reflux under nitrogen for 3 h. The mixture was cooled to room temperature and purified by preparative HPLC (C-18 reverse phase column, MeOH/water, 1:9) to give **5** (3.4 g, 85%)

2-Amino-7-propyl-9-(β-D-ribofuranosyl)purine-6,8(1H)-dione (8). A mixture of **5** (1g, 2.9 mM), 10% Pd/C (100 mg), and ethanol (100 mL) was stirred at room temperature under an atmosphere of hydrogen for 3 h. The resulting mixture was filtered through a pad of Celite and washed with ethanol (100 mL). The combined filtrates were concentrated under vacuum. The residue was dissolved in methanol (20 mL) and treated with ether (200 mL). The solid which formed was filtered and dried at 60°C in a vacuum oven to give **8** (0.56 g, 55%), m.p. 151-153°C. IR (KBr) 1680, 1640 cm^{-1}. ^1H NMR (90 MHz) δ 10.80 (bs, 1H, exchangeable, CONH), 6.5 (bs, 2H, exchangeable, NH_2), 5.50 (d, J=6 Hz, 1H, C_1'), 0.9 (t, J=7 Hz, 3H, CH_3). Anal. Calcd. for $C_{13}H_{19}N_5O_6$: C, 45.75; H, 5.61; N, 20.56. Found: C, 45.41; H, 5.56; N, 20.09.

2-Amino-7-butyl-9-(β-D-ribofuranosyl)purine-6,8(1H)-dione (9). The general procedure was followed with butyl iodide as the alkylating agent to afford **9** (15% yield), mp >230°C. ^{1}H NMR (90 MHz) δ 10.8 (bs, 1H, exchangeable, CONH), 6.4 (bs, 2H, exchangeable, NH_2), 5.6 (d, J=6 Hz, 1H, C_1'), 0.9 (t, J=6 Hz, 3H, CH_3). Anal. Calcd. for $C_{14}H_{21}N_5O_6$: C, 47.32, H, 5.96; N, 19.71. Found: C, 47.03; H, 5.86; N, 19.56.

2-Amino-7-*E*-(2-butenyl)-9-(β-D-ribofuranosyl)purine-6,8(1H)-dione Hydrate (10). The general procedure was followed with *E*-(2-butenyl) bromide as the alkylating agent to afford **10** (10% yield), mp 167-170°C. ^{1}H NMR (90 MHz) δ 10.7 (bs, 1H, exchangeable, CONH), 6.4 (bs, 2H, exchangeable, NH_2), 5.5 (overlapping peaks, 3H, C_1' and vinyl protons), 1.5 (d, J=3 Hz, 3H, CH_3). Anal. Calcd. for $C_{14}H_{19}N_5O_6 \cdot H_2O$: C, 42.58, H, 5.70; N, 18.86. Found: C, 45.16; H, 5.61; N, 18.82.

2-Amino-7-hexyl-9-(β-D-ribofuranosyl)purine-6,8(1H)-dione (11). The general procedure was followed with hexyl iodide as the alkylating agent to afford **11** (8% yield), mp 196-199°C. ^{1}H NMR (90 MHz) δ 10.8 (bs, 1H), 6.5 (bs, 2H), 5.6 (d, J=6 Hz, 1H, C_1'), 0.9 (t, J=6 Hz, 3H, CH_3) . Anal. Calcd. for $C_{16}H_{25}N_5O_6$: C, 50.12, H, 6.57; N, 18.27. Found: C, 49.93; H, 6.52; N, 18.24.

2-Amino-7-(2-propenyl)-9-[β-D-ribofuranosyl-2',3'-(isopropylidene)]-purine-6-one-8(1H)-thione 0.5Hydrate (12). A solution of 7-(2-propenyl)-8-thioxoguanosine[10] (6 g, 16.9 mmol), 2,2-dimethoxypropane (5 mL, 40.7 mM), and concentrated H_2SO_4 (10 drops) in acetone (200 mL) was stirred under nitrogen for 52 h. The mixture was cooled to 0°C and treated with concentrated NH4OH (5 mL). Most of the solvent was removed and the white solid was filtered. The solid was washed with water, acetone, and ether, and then dried at 60°C under vacuum to afford **12** (5.0 g, 75%), mp 237°C (dec). ^{1}H NMR (90 MHz) δ 10.8 (bs, 1H), 6.95 (bs, 2H), 6.58 (bs, 1H), 5.92 (m, 1H), 1.52 (s, 3H). Anal. Calcd. for $C_{16}H_{21}N_3O_3S \cdot 0.5H_2O$: C, 47.51, H, 5.48; N, 17.32. Found: C, 47.08; H, 5.12; N, 17.30.

2-Amino-7-(2-propenyl)-9-[β-D-ribofuranosyl-2',3'-(cyclohexylidene)]purine-6,8(1H)-dione 0.5Hydrate (13). A solution of **5** (1 g, 2.97 mmol), cyclohexanone (0.60 mL, 6.0 mmol), triethyl orthoformate (0.72 mL, 4.4 mmol), and saturated HCl in dioxane (1.4 mL) in DMF (12 mL) was stirred at ambient temperature for 3 d. The reaction mixture was then poured into ether (175 mL). The solvent was decanted, and the residue was dissolved into $CHCl_3$ and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$, and the combined organics were dried ($MgSO_4$), filtered, and concentrated. This material was purified by chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH, 90:9:1), and dried at 48°C overnight under vacuum to give **13** (290 mg, 23%), mp 209-211°C. ^{1}H NMR (400 MHz) δ 10.8 (bs, 1H), 6.5

(bs, 2H), 5.9 (m, 1H), 5.8 (s, 1H), 5.28 (d, 1H), 4.9-5.1 (m, 3H), 4.8 (t, 1H, exchangeable), 4.4 (d, 2H), 3.95 (m, 1H), 3.4-3.5 (m, 2H), 1.2-1.7 (m, 10H). Anal. Calcd. for $C_{19}H_{25}N_5O_6 \cdot H_2O$: C, 53.54, H, 6.00; N, 16.43; H_2O, 1.58. Found: C, 53.23; H, 6.07; N, 16.23; H_2O, 1.54.

2-Amino-7-(2-propenyl)-9-[β-D-ribofuranosyl-2',3'-(tetrahydro-4H-pyran-4-one)]purine-6,8(1H)-dione (14). A solution of **5** (1 g, 2.97 mmol), tetrahydro-4H-pyran-4-one (0.7 mL, 7.6 mmol), triethyl orthoformate (1.36 mL, 6.0 mmol), and saturated HCl in dioxane (0.5 mL) in DMF (4 mL) was stirred at ambient temperature for 3 d. The reaction mixture was then poured into ether (500 mL). The solvent was decanted, and the residue was dissolved into $CHCl_3$ and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ (4X), and the combined organics were dried ($MgSO_4$), filtered, and concentrated. This material was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 93:7), and recrystallized from MeOH/ether/pentane and dried at 60°C overnight under vacuum to give **14** (80 mg, 6%), mp 170-174°C. ^1H NMR (400 MHz) δ 10.97 (bs, 1H), 6.6 (bs, 2H), 5.9 (m, 1H), 5.83 (s, 1H), 5.32 (d, 1H), 5.11 (d, 1H), 5.0 (m, 2H), 4.8 (t, 1H), 4.39 (d, 2H), 4.00 (m, 1H), 3.4-3.7 (m, 6H), 1.8 (br s, 2H), 1.65 (br s, 2H). Anal. Calcd. for $C_{18}H_{23}N_5O_7$: C, 51.30, H, 5.50; N, 16.62. Found: C, 51.60; H, 5.74; N, 16.15.

2-Amino-7-(2-propenyl)-9-[5'-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-purine-6,8(1H)-dione Hydrate (15). A solution of **5** (1.0 g, 2.96 mmol), imidazole (0.22 g, 3.26 mmol), and t-butyldimethylsilyl chloride (0.5 g, 3.3 mmol) in pyridine (5 mL) was allowed to stir at ambient temperature. After 6 h, water (25 mL) was added to the reaction, and the resultant white solid was collected and washed 3X with ether. The product was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH, 90:9:1) followed by recrystallization from MeOH/EtOAc to give **15** as a white solid (0.60 g, 45%), mp 257-259°C. ^1H NMR (400 MHz) δ 10.87 (br s, 1H), 6.5 (br s, 2H), 5.9 (m, 1H), 5.60 (s, 1H), 5.3 (s, 1H), 5.12 (d, 1H), 5.04 (d, 1H), 4.97 (d, 1H), 4.88 (m, 1H), 4.41 (br s, 2H), 4.18 (m, 1H), 3.8 (br m, 2H), 3.62 (m, 1H), 0.88 (s, 9H). $[\alpha]_D^{25}$ = -2.0° (c 1.0, MeOH). Anal. Calcd. for $C_{19}H_{31}N_5O_6Si$: C, 50.31; H, 6.89; N, 15.44. Found: 50.37; H, 6.77; N, 15.57.

2-Amino-7-(2-propenyl)-9-(β-D-ribofuranosyl-2',3'-sulfite)purine-6,8(1H)-dione 0.25Hydrate (16). A solution of **15** (0.50 g, 1.1 mmol) in pyridine (5 mL) was chilled to 0°C and then treated with $SOCl_2$ (0.16 mL, 2.2 mmol). After stirring for 1 h, MeOH (ca. 1.5 mL) was added, and the solution was then treated with tetrabutylammonium fluoride (4 mL of a 1M solution in THF). After stirring overnight, an additional 4 mL of the fluoride reagent was added. The solvents were mostly evaporated in vacuo, and the pale yellow residue was treated with water (ca. 12 mL). After 1 h with

cooling, the solids were filtered and washed with isopropanol and ether and then air-dried to give a solid which was recrystallized from DMSO/water to give 0.22 g of a white powder, dried in vacuo at room temperature for two days (51%, m.p. 275-278°C). ^{1}H NMR (400 MHz) δ 10.9 (br s, 1H), 6.7 (br s, 2H), 6.11 (br d, 0.77H, major diastereomer), 6.07 (d, 0.23H, minor diastereomer), 5.9 (m, 2.77H), 5.7 (m, 0.23H), 5.0-5.12 (m, 3H), 4.4 (br s, 2H), 4.01 (t, 2H), 3.5-3.6 (m, 2H). $[\alpha]_D^{25}$ = 6.2° (c 1.0, DMSO). Analysis of the ^{1}H NMR spectrum revealed a 3.4:1 mixture of exo:endo sulfite diastereomers. Anal. Calcd. for $C_{13}H_{15}N_5O_7S \cdot 0.25H_2O$: C, 40.05; H, 4.00; N, 17.96; H_2O, 1.15. Found: C, 40.02; H, 3.92; N, 17.68; H_2O, 0.95.

2-Amino-7-(2-propenyl)-9-(β-D-ribofuranosyl-2',3'-sulfate)purine-6,8(1H)-dione (17). A solution of **15** (3.55 g, 7.8 mmol), sulfuryldiimidazole (1.55 g, 7.8 mmol), LiH (0.16 g, 20.1 mmol) and DMF (1.6 mL) in pyridine (16 mL) was allowed to stir under a argon atmosphere for 4.5 h. Since some of the starting guanosine material was still present by TLC, an additional 50 mg of LiH was added. After a total of 7h, the solution was cooled and treated with HOAc (15 mL). After stirring for 15 min, the reaction mixture was added dropwise to ca. 200 mL of water, with vigorous stirring. The pale orange solid was collected and washed with water and allowed to air dry overnight. The product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH; 95:4.5:0.5) to give 1.68 g (42%) of a white foamy glass. High-field ^{1}H NMR and mass spectral analysis supported the structure as being the 5'-(t-butyldimethylsilylether) of **17**. A sample of this substance (1.10 g, 2.13 mmol) was dissolved in MeOH/CH_2Cl_2 (1:1, 10 mL). This solution was filtered, and then the solution was treated with p-TsOH hydrate (0.40 g, 2.13 mmol) and stirred. After 1 h, the solution was chilled in an ice/water bath. The crystals that formed were collected and washed with cold MeOH and ether. The resultant solid (0.72 g, 85%) was air-dried and then dried under vacuum at room temperature to give **17** (m.p. >190 °C with decomposition). ^{1}H NMR (400 MHz) δ 11.0 (br s, 1H), 6.7 (br s, 2H), 6.32 (s, 1H), 5.9-6.1 (br m, 3H), 5.05-5.15 (br m, 3H), 4.4 (br m, 3H), 3.6 (m, 3H). $[\alpha]_D^{25}$ = -14.8° (c 0.4, DMSO). Anal. Calcd. for $C_{13}H_{15}N_5O_8S$: C, 38.90; H, 3.77; N, 17.45. Found: C, 39.23; H, 3.86; N, 17.27.

Biological Methods. CBA/CaJ and C57BL/6J mice, 8 to 12 weeks of age, were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were maintained on Wayne Lab-blox F6 pellets (Allied Mills, Inc., Chicago, IL) and chlorinated water acidified with HCl to a pH of 3.0. The culture medium was RPMI 1640 medium (Flow Laboratories Inc., Rockville, MD) supplemented with glutamine, sodium pyruvate, nonessential amino acids, penicillin, streptomycin, and 5% fetal calf serum. For evaluation of the primary humoral response, 5 X 10^6 to 1 X 10^7 murine spleen cells, prepared as

described previously,[19] were cultured in 1 mL culture medium containing sheep red blood cells (SRBC) as an immunogen. Cultures were incubated for 4 to 5 days at 37°C in 5% CO_2. Quantitation of the number of cells actively secreting antibodies was carried out using a modification[20] of the hemolytic plaque assay of Jeme and Nordin[21]. Cultured cells were suspended in agarose containing SRBC (Colorado Serum Co., Denver, CO) and were incubated for 1 h at 37°C in SRBC-adsorbed guinea pig complement following a 1.5 h incubation without complement.

Acknowledgments. We acknowledge the assistance and encouragement of David Anderson, Catherine Lau, and Thomas Shultz of the R. W. Johnson Pharmaceutical Research Institute. Chemical Development support was provided by Donald Barton, Judith Cohen, Charles Kasulanis, and Jeffrey Grimm. We appreciate the assistance of Robert Capetola, William J. Hennen, and Roland K. Robins at various stages in the project.

REFERENCES

1. (a) Bonnet, P. A.; Robins, R. K. *J. Med. Chem.* **1993**, *36*, 635-653. (b) St. Georgiev, V. *Med. Res. Rev.* **1990**, *10*, 371-409. (c) Hadden, J. W. *J. Am. Med. Assoc.* **1987**, *258*, 3005-3010.
2. (a) Hadden, J. W. *Trends Pharm. Sci.* **1993**, *14*, 169. (b) Failli, A.; Caggiano, T. J. *Curr. Opin. Ther. Pat.* **1992**, *2*, 882-892. (c) Werner, G. H. *Drugs Today* **1990**, *26*, 269-277.
3. (a) Skulnick, H. I.; Weed, S. D.; Eidson, E. E.; Renis, H. E.; Wierenga, W.; Stringfellow, D. A. *J. Med. Chem.* **1985**, *28*, 1864-1869. (b) Skulnick, H. I.; Ludens, J. H.; Wendling, M. G.; Glenn, E. M.; Rohloff, N. A.; Smith, R. J.; Wierenga, W. *J. Med. Chem.* **1986**, *29*, 1499-1504. (c) For a recent report of a study using bropirimine, see: Borden, E. C.; Sidky, Y. A.; Erturk, E.; Wierenga, W.; Bryan, G. T. *Cancer Res.* **1990**, *50*, 1071-1074.
4. Holmes, R. E.; Robins, R. K. Purine Nucleosides. VII. *J. Am. Chem. Soc.* **1964**, *86*, 1242-1245.
5. Goodman, M. G.; Weigle, W. O. *Proc. Natl. Acad. Sci., U.S.A.* **1981**, *78*, 7604-7608.
6. (a) Goodman, M. G.; Hennen, W. J. *Cell. Immunol.* **1986**, *102*, 395-402. (b) Rizkalla, B. H.; Robins, R. K.; Broom, A. D. *Biochim. Biophys. Acta* **1969**, *195*, 285-293. (c) An improved synthesis of **3** has appeared: Kini, G. D.; Hennen, W. J.; Robins, R. K. *Nucleosides Nucleotides* **1987**, *6*, 581-587.
7. (a) Nagahara, K.; Anderson, J. D.; Kini, G. D.; Dalley, N. K.; Larson, S. B.; Smee, D. F.; Jin, A.; Sharma, B. S.; Jolley, W. B.; Robins, R. K.; Cottam, H. B. *J. Med.*

Chem. **1990**, *33*, 407-415. (b) Kini, G. D.; Anderson, J. D.; Sanghvi, Y. S.; Lewis, A. F.; Smee, D. F.; Revankar, G. R.; Robins, R. K.; Cottam, H. B. *J. Med. Chem.* **1991**, *34*, 3006-3010.

8. Goodman, M. G.; Weigle, W. O. *J. Immunol.* **1983**, *130*, 2042.
9. Portions of the work described here are found in US Patent 5,011,828 (3 April 1991).
10. (a) Anderson, D. W.; Capetola, R. J. in "Gamete Interaction: Prospects for Immunocontraception"; Alexander, N. J., Ed.; Wiley-Liss: New York, 1989; pp 523-548. (b) Pope, B. L.; Chourmouzis, E.; Sigindere, J.; Capetola, R. J.; Lau, C. Y. *Int. J. Immunopharm.* **1992**, *14*, 1375-1382.
11. Come, J.; Burr, L.; Chen, R. *Tetrahedron Lett.* **1991**, *32*, 4823-4826.
12. Seela, F.; Waldek, S. *Nuc. Acids Res.* **1975**, *2*, 2343-2354.
13. (a) Staab, H. A.; Wendel, K. *Annalen* **1966**, *694*, 86-90. (b) Tewson, T. J.; Soderlind, M. *J. Carbohydr. Chem.* **1985**, *4*, 529-543. (c) We thank Dr. Michael Costanzo for a gift of sulfuryl diimidazole.
14. Hansch, C.; Bjorkroth, J. P.; Leo, A. *J. Pharm. Sci.* **1987**, *76*, 663.
15. Sanger, W. "Principles of Nucleic Acid Structure"; Springer-Verlag: New York, 1984; pp 21-78.
16. Tavale, S. S.; Sobell, H. M. *J. Mol. Biol.* **1970**, *48*, 109-123.
17. Uesugi, S.; Ikehara, M. *J. Am. Chem. Soc.* **1977**, *99*, 3250-3253.
18. Katze, J. R. *Proc. Soc. Expl. Biol. Med.* **1985**, *179*, 492-496.
19. Goodman, M. G.; Fidler, J. M.; Weigle, W. O. *J. Immunol.* **1978**, *121*, 1905-1913.
20. Goodman, M. G.; Weigle, W. O. *J. Immunol.* **1985**, *135*, 3284-3288.
21. Jeme, N. K.; Nordin, A. A. *Science* **1963**, *140*, 405-407.

Received 8/2/93
Accepted 10/18/93