4c, 123206-09-7; (±)-(R*,R*)-5a, 123206-10-0; (±)-(R*,S*)-5a, 123206-11-1; 5b, 106746-07-0; 5c, 123206-12-2; (±)-(R*,R*)-6a, 102614-00-6; (±)-(R*,S*)-6a, 102614-01-7; 6b, 106746-09-2; 6c, 123206-13-3; 7a, 105108-49-4; 7b, 123206-14-4; 7c, 41036-61-7; 7d, 63769-88-0; 7e, 63769-98-2; 7f, 38675-10-4; 8a, 123206-15-5; 8b, 123206-16-6; 8c, 106771-14-6; 8d, 102614-06-2; 8e, 106771-19-1; 8f, 123206-17-7; 8g, 123239-02-1; (R)-9a, 123206-18-8; (S)-9a, 123285-38-1; (R)-9b, 123239-03-2; (S)-9b, 123286-93-1; (R)-9c, 123285-39-2; (S)-9c, 123285-40-5; (R)-9d, 123285-41-6; (S)-9d, 123285-42-7; (R)-9e, 123285-43-8; BOC-deblocked (R)-9e·HCl, 123285-58-5; (S)-9e, 123285-44-9; BOC-deblocked (S)-9e·HCl, 123285-59-6; (R)-9f, 123206-19-9; (S)-9f, 123285-45-0; (R)-9g, 123206-20-2; (S)-9g, 123285-46-1; (R)-9h, 123206-21-3; (S)-9h, 123285-47-2; (R)-9i, 123206-22-4; (S)-9i, 123285-48-3; (R)-10, 123285-49-4; (S)-10, 123285-50-7; 11, 15761-39-4; 12, 123239-04-3; 13, 123239-05-4; 14, 123206-23-5; 15, 123206-24-6; (R)-16, 123206-25-7; (S)-16, 123285-51-8; 17a, 105499-11-4; 17b, 123285-52-9; 17c, 98818-36-1; 18a, 565-81-1; 18b, 117213-88-4; 18c, 62084-21-3; 19a, 106746-17-2; 19a·HCl, 123206-26-8; 19b, 123206-27-9; 19b·HCl, 123206-28-0; 19c, 123206-29-1; 19c·HCl, 123206-30-4; 20, 106771-16-8; (R)-21, 123285-53-0; (S)-21, 123285-54-1; 22, 123206-31-5; 23, 123206-32-6; 24, 123206-33-7; (R)-25, 123206-34-8; (S)-25, 123285-55-2; 26, 106771-28-2; 27, 106785-91-5; (R)-28, 123285-56-3; (S)-28, 123285-57-4; BOC-Val-OH, 13734-41-3; H-Pro-OBzl·HCl, 16652-71-4; BOC-Val-Pro-OBzl, 58872-03-0; H-Val-Pro-OBzl·HCl, 95501-60-3; MeO-Suc-OH, 3878-55-5; MeO-Suc-Val-Pro-OBzl, 123206-35-9; $MeOCOCH_2CH_2COCl$, 1490-25-1; Dan-Cl, 605-65-2; N^{α}-$AdSO_2$-N^{ϵ}-Pht-L-Lys-OH, 98385-08-1; $AdSO_2$-Lys-Pro-NHCH(*i*-Pr)$CH(OH)CF_3$, 123206-36-0; $BrF_2CCOOEt$, 667-27-6; BOC-NHCH(*i*-Pr)$CH(OH)CF_2COOEt$·HCl, 123206-37-1; elastase, 9004-06-2; cathepsin G, 56645-49-9.

Supplementary Material Available: Tables listing kinetic constants for some of the elastase inhibitors synthesized (9c-g,i, 10, 16, 21, 23, and 28) and analysis data for 4a-c, 5b, 6a,b, 8b,f, 9a, 10, 18b,c, 23, 26, 28, and MeO-Suc-Val-Pro-OBz (3 pages). Ordering information is given on any current masthead page.

Thiazolo[4,5-*d*]pyrimidine Nucleosides. The Synthesis of Certain 3-β-D-Ribofuranosylthiazolo[4,5-*d*]pyrimidines as Potential Immunotherapeutic Agents

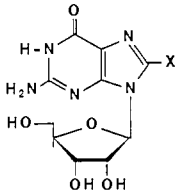
Katsuhiko Nagahara,[†,‡] Jack D. Anderson, Ganesh D. Kini, N. Kent Dalley,[†] Steven B. Larson, Donald F. Smee, Ai Jin, Brahma S. Sharma, Weldon B. Jolley, Roland K. Robins, and Howard B. Cottam*

ICN Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, California 92626, and Department of Chemistry, Brigham Young University, Provo, Utah 84602. *Received March 13, 1989*

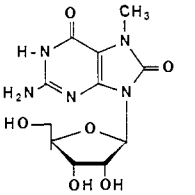
Novel analogues of the naturally occurring purine nucleosides were synthesized in the thiazolo[4,5-*d*]pyrimidine ring system to determine the immunomodulatory effects of insertion of a sulfur atom in place of nitrogen at position 7 of the purine ring. In particular, 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (7, guanosine analogue), 3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,5,7(3*H*,4*H*,6*H*)-trione (8, xanthosine analogue), 3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (10, inosine analogue), and 7-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (32, adenosine analogue) were prepared, as well as the 8-mercaptoguanosine (14) and 6-mercaptoguanosine (17) analogues. Single-crystal X-ray studies confirmed the structural assignment of 17 and 32 as having the β-configuration with the site of glycosylation at N3. The nucleosides were evaluated for their ability to potentiate various murine immune functions in direct comparison to the known active agents 8-bromoguanosine (1), 8-mercaptoguanosine (2), and 7-methyl-8-oxoguanosine (3). Two of the guanosine analogues, 7 and 14, were found to exhibit significant immunoactivity relative to the positive control compounds (1-3), while the adenosine, inosine, xanthosine, and 6-mercaptoguanosine analogues were devoid of activity. Compound 7 exhibited greater immunoactivity than any of the other guanosine analogues and derivatives in all test systems. Specifically, 7 was shown to be about twice as potent as 3 in the murine spleen cell mitogenicity assay. In addition, treatment with 7 produced about a 4-fold increase in natural killer cell cytotoxicity, while treatment with 3 afforded a 3-fold increase over controls. Finally, 7 provided excellent protection (92% survivors compared to 0% for placebo controls) against Semliki Forest virus in mice. Induction of interferon may account for the major mode of action of these guanosine analogues.

The development of clinically useful agents for enhancing host resistance to disease and restoring impaired immune functions has become a major objective of current pharmaceutical research efforts. Indeed, the phenomenal growth in basic understanding of the immune system over the past 15 years and the demonstration of the prevalence of cellular immune defects in cancer, aging, autoimmunity, and infectious diseases have led to the development of a variety of specific and nonspecific immunotherapeutic agents.[1,2]

While many of the naturally occurring cytokines which potentiate the immune response are large glycoproteins, several synthetic small molecules have been shown to modulate immune functions as well.[3] Certain ribonucleosides of guanine substituted at C8 have been extensively studied primarily as modulators of B-cell activation.[4] The most active and most studied derivatives include 8-bromoguanosine (1, first prepared in our labo-



1, X = Br
2, X = SH



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† Brigham Young University.
‡ Present address: School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane Minato-KU, Tokyo 108, Japan.

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ratory in 1964[5]), 8-mercaptoguanosine[5] (2), and 7-methyl-8-oxoguanosine (3, synthesized in our laboratory in 1969[6] and later submitted to the Scripps Research group in a collaborative research program). These low molecular weight guanosines have been reported to act as intracellular mitogens in murine splenic B lymphocytes[7] and to augment the proliferation and differentiation of murine T cells in the presence of other stimulatory signals.[8,9] More recently, 1 was shown to activate murine NK cells and macrophages by inducing the production of interferon.[10]

The ability of 3 to initiate lymphocyte activation in murine spleen cell cultures has been demonstrated.[11] At optimal concentrations, the peak response evoked by 3 is reported to be about twice the magnitude of that exhibited by 1 and 2. The two molecular features, the 7-methyl function and the 8-oxo group, are both required for this level of activity. Furthermore, both 2 and 3 augment the humoral response of murine splenic lymphoid cells in vitro when cultured in the presence of sheep red blood cells.[11] But while each nucleoside enhances the response to about the same level, 3 exhibits a concentration optimum 10-fold lower than that required for 2. Thus, 7-methyl-8-oxoguanosine is a more effective B-cell mitogen and a more potent adjuvant for primary humoral immune responses than either 8-bromo- or 8-mercaptoguanosine.[11] These results prompted us to evaluate a novel guanosine analogue in which the *N*-methyl function at position 7 of 3 is replaced by a sulfur atom, resulting in the thiazolo[4,5-*d*]pyrimidine ring system.

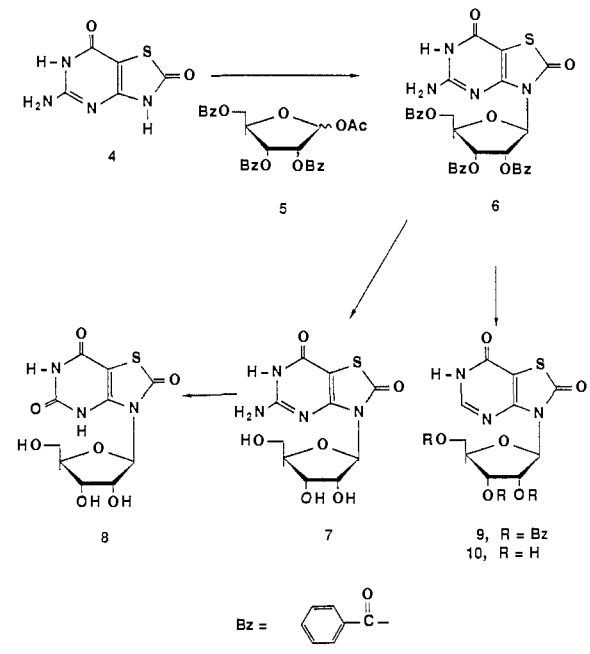
In order to investigate the qualitative structure–activity relationships with respect to immunological properties, the xanthosine, inosine, and adenosine analogues were also synthesized in this thiazolo[4,5-*d*]pyrimidine ring system. The target compounds were all tested for immunomodulation in screening systems designed to detect both immunopotentiation as well as immunosuppression of various components of the immune response.

Chemistry

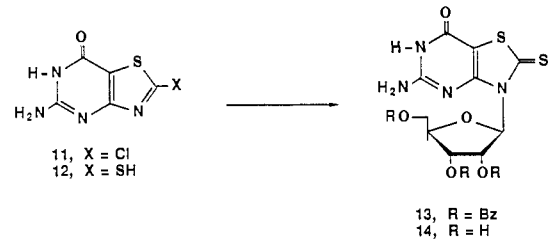
The most straightforward approach to the synthesis of the guanosine analogue 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione appeared to be by the direct glycosylation of the preformed guanine base analogue by the general procedure of Vorbrüggen and co-workers[12] (Scheme I). Thus, 5-aminothiazolo[4,5-*d*]-pyrimidine-2,7(3*H*,6*H*)-dione (4), prepared in five steps from the commercially available 2,4-diamino-6-hydroxypyrimidine by the method of Baker and Chatfield,[13] was glycosylated by initial trimethylsilylation using hexamethyldisilazane followed by treatment with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (5) in the presence of trimethylsilyl trifluoromethanesulfonate as a catalyst. The major product, 5-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (6), was isolated in 77% yield. Treatment of 6 with sodium methoxide in methanol gave the deprotected guanosine analogue 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (7) in 78% yield. When 7 was deaminated with excess nitrous acid, the xanthosine analogue 3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,5,7-(3*H*,4*H*,6*H*)-trione (8) was produced in moderate yield. Replacement of the 5-amino group of compound 6 by a hydrogen atom was accomplished by treatment of 6 with *tert*-butyl nitrite in tetrahydrofuran to yield 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (9). Deprotection of 9 using sodium methoxide in methanol or methanolic ammonia provided the inosine analogue 3-β-D-ribofuranosylthiazolo[4,5-*d*]-pyrimidine-2,7(3*H*,6*H*)-dione (10) in good yield.

Since the immunostimulating properties of 8-mercaptoguanosine (2) were so well documented, we undertook to prepare the analogous nucleoside in the thiazolo[4,5-*d*]pyrimidine system. The most successful approach to this compound is depicted in Scheme II, starting with 5-amino-2-chlorothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (11).[13] Compound 11 was treated with NaSH in ethylene glycol at 110 °C to provide 5-amino-2,3-dihydro-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (12), in good yield. Glycosylation of 12 by the same procedure as that used to prepare the 2-oxo compound 6 (except that some heating was employed to ensure that any putative *S*-glycoside formed would be converted to the more thermodynamically stable *N*-glycoside) resulted in the formation of 5-amino-2,3-dihydro-2-thioxo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-7-(6*H*)-one (13). Treatment of 13 with sodium methoxide in methanol yielded the 8-mercaptoguanosine analogue 5-amino-2,3-dihydro-2-thioxo-3-β-D-ribofuranosyl-

Scheme I



Scheme II



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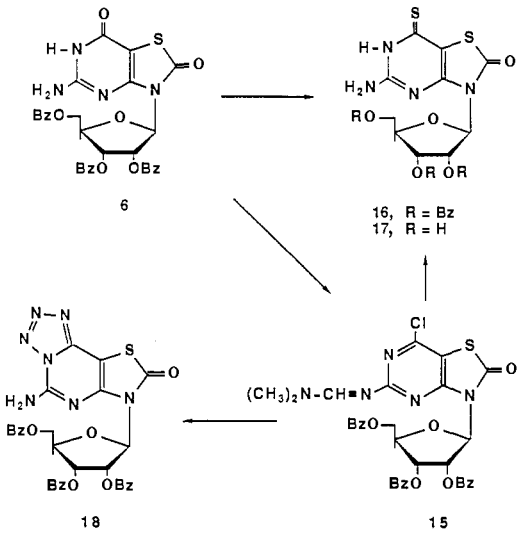
(12) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1980**, *114*, 1234.
(13) Baker, J. A.; Chatfield, P. V. *J. Chem. Soc. C* **1970**, 2478.

Scheme III



thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**14**), in good yield. Verification of the structural assignment of **14** was accomplished by converting a small sample of **14** to compound **7** by treatment of **14** with alkaline hydrogen peroxide at 60 °C for 30 min. The resulting product was identical with **7** as judged by reversed-phase HPLC and UV spectral data.

Various related derivatives in the guanosine analog series were also prepared. The 6-thioguanosine analogue was prepared by two routes starting from **6** (Scheme III). In the first approach, **6** was treated with the mild chlorinating agent dimethyl(chloromethylene)ammonium chloride[14] (generated in situ from thionyl chloride and DMF), which provided 5-[[(*N*,*N*-dimethylamino)methylene]amino]-7-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo-[4,5-*d*]pyrimidin-2(3*H*)-one (**15**) in 84% yield. Reaction of **15** with thiourea in refluxing ethanol gave the protected thioguanosine analogue 5-amino-6,7-dihydro-7-thioxo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (**16**) in good yield. It was later found that compound **16** could also be prepared directly from **6** by reaction with P_2S_5 in pyridine. Deprotection of **16** was accomplished either with sodium methoxide in methanol or with methanolic ammonia to give the 6-thioguanosine analogue 5-amino-6,7-dihydro-7-thioxo-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (**17**), as the crystalline monohydrate in 87% yield. The structure of **17** was confirmed by single-crystal X-ray analysis as described below (Figure 1).

In an attempt to prepare the 5,7-diamino derivative, the chloro function at position 7 of **15** was also nucleophilically substituted by azide with sodium azide in dry DMF, which subsequently ring closed at N6 to form the new tricyclic ring compound 5-amino-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazolo[5,1-*f*]thiazolo[4,5-*d*]pyrimidin-8-(7*H*)-one (**18**), in 67% yield. However, suitable reducing agents to convert the tetrazole (azide) function to an amino group without resulting in concomitant decomposition were not found.

In an effort to study the thiazolo[4,5-*d*]pyrimidine ring system with respect to the order of nucleophilic substitution at the 2-, 5-, and 7-positions and possibly use this information to synthesize the adenosine analogue, we chlorinated the readily available 2-chlorothiazolo[4,5-*d*]-

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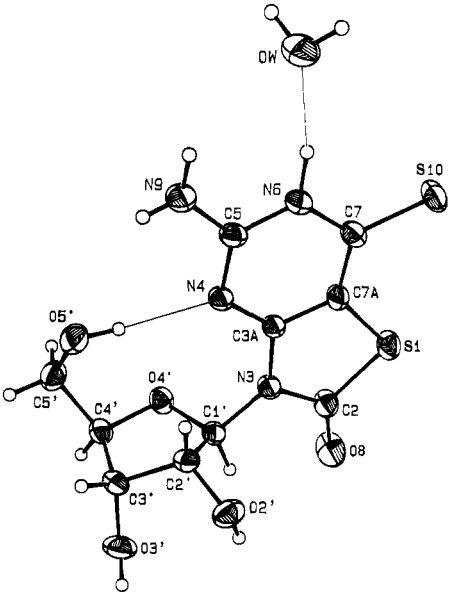
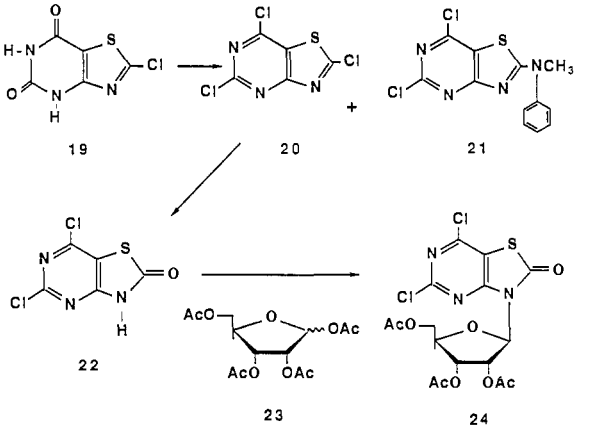


Figure 1. Perspective drawing of compound **17** illustrating atom labelling and molecular conformation. Selected hydrogen bonds are indicated by thin lines. Thermal ellipsoids are drawn at the 50% probability level.

Scheme IV



pyrimidine-5,7(4*H*,6*H*)-dione (**19**)[13] with refluxing $POCl_3$ and *N*,*N*-dimethylaniline (Scheme IV). A 74% yield of the desired 2,5,7-trichlorothiazolo[4,5-*d*]pyrimidine (**20**) was obtained along with a small amount of 5,7-dichloro-2-(*N*-methylanilino)thiazolo[4,5-*d*]pyrimidine (**21**). The structure of **21** was assigned unequivocally by single-crystal X-ray analysis and is detailed elsewhere.[15] Trichloro compound **20** was carefully hydrolyzed in 1 N NaOH at 60 °C in order to obtain the monooxo derivative 5,7-dichlorothiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (**22**), the structure of which was verified by single-crystal X-ray analysis.[16] Thus, it appears that nucleophilic substitution in the trichloro derivative occurs first at the 2-position and then at the 7- or 5-position, under the neutral or basic conditions employed here.

Reaction of **22** with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (**23**) under fusion glycosylation conditions produced an excellent yield of 5,7-dichloro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (**24**). Attempts to use **24** for further modification by nucleophilic

(15) Larson, S. B.; Cottam, H. B.; Robins, R. K. *Acta Crystallogr.* In press.

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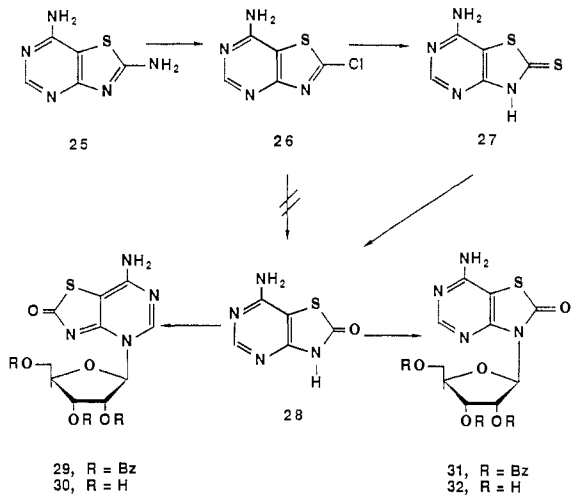
Scheme V



substitution to obtain the adenosine analogue were unsuccessful even under very mild conditions. This was likely due to the labile nature of the thiazole ring toward nucleophilic ring-opening, although no attempts were made to isolate and characterize the products of these reactions. The various nucleophiles evaluated were azide, ammonia, and hydrazine, as well as methoxide, hydroxide, and thiourea.

These unsuccessful attempts at nucleophilic substitution prompted us to investigate the synthesis of the adenosine analogue from its preformed heterocycle in the same manner as that used to obtain the guanosine analogue. The reported 2,7-diaminothiazolo[4,5-*d*]pyrimidine (**25**)[13] served as the starting material for our studies (Scheme V). Treatment of **25** with nitrous acid under conditions similar to those used to prepare **11**[13] provided 7-amino-2-chlorothiazolo[4,5-*d*]pyrimidine (**26**). The structure of compound **26** was verified by single-crystal X-ray analysis.[17] Attempts, however, to convert the chloro function directly to oxo were unsuccessful. Treatment of **26** with NaSH in DMF at 0 °C yielded the 2-mercapto derivative 7-aminothiazolo[4,5-*d*]pyrimidine-2(3*H*)-thione (**27**), in good yield. The conversion of the 2-thioxo function in **27** to an oxo function was accomplished with cold alkaline hydrogen peroxide to yield 7-aminothiazolo[4,5-*d*]pyrimidin-2-(3*H*)-one (**28**). Reaction of **28** with benzoyl-protected sugar **5** under the same glycosylation conditions (at room temperature) as used to produce the blocked guanosine analogue **6** resulted in the formation of an unexpected product, presumed to be the blocked 4-ribofuranosyl isomer 7-amino-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo-[4,5-*d*]pyrimidin-2-one (**29**), as the only isomer detected and isolated. When, however, the same reaction was carried out at elevated temperature (80 °C), the predominant product obtained was the desired 3-ribofuranosyl isomer 7-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (**31**). Both isomers **29** and **31** were deprotected with sodium methoxide in dry methanol to obtain presumably 7-amino-4-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2-one (**30**) and 7-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2-(3*H*)-one (**32**), respectively. The assignment of the site of glycosylation for both **30** and **32** was based primarily on ultraviolet spectra in which **30** (the presumed N-4 isomer) displayed absorption maxima at longer wavelengths in pH 1 buffer compared to the aglycon **28**, whereas **32** (the N3

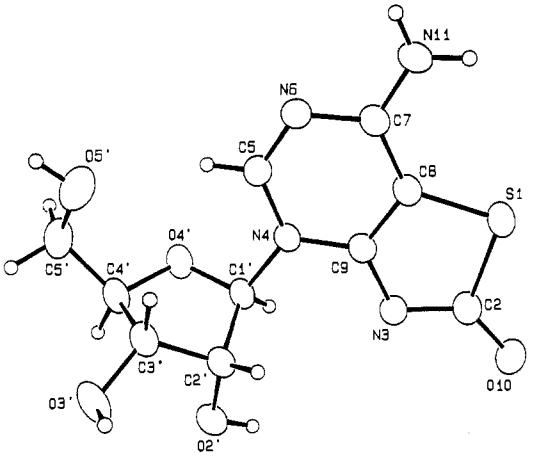


Figure 2. Perspective drawing of compound **30** indicating anomeric configuration and site of glycosylation. Thermal ellipsoids are drawn at the 50% probability level.

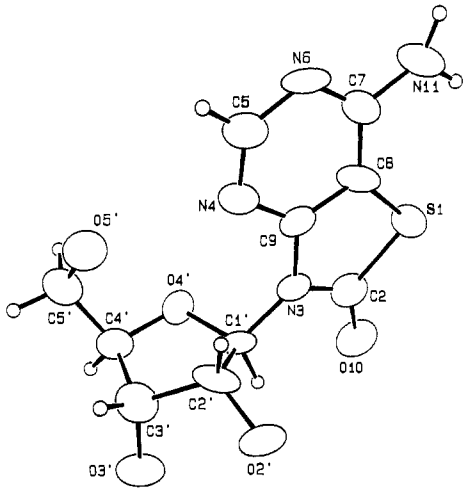


Figure 3. Perspective drawing of compound **32** indicating anomeric configuration and site of glycosylation. Thermal ellipsoids are drawn at the 50% probability level.

isomer) possessed a spectrum almost identical with that of **28** at pH 1 and a hypsochromic shift at pH 7 and 11 relative to the aglycon. It has been shown previously for 7-deazapurines[18-20] and for purines[21] that substitution at N9 (purine numbering, analogous to **32**) results in a slight hypsochromic or no shift relative to the aglycon whereas substitution at N3 (purine numbering, analogous to **30**) leads to a bathochromic shift relative to the aglycon. The observed relative bathochromic shifts of 11 nm for **30** at pH 1 and 20 nm at pH 7 and 11 lend support to the structural assignment as the N4 isomer. In addition, single-crystal X-ray diffraction studies confirmed the structural assignments of **30** as the N4 β isomer and **32** as the N3 β isomer (Figures 2 and 3), the details of which will be reported elsewhere.[22]

X-ray Crystallographic Studies. Since a suitable crystal of **7** was not available, a derivative of **7**, the 6-

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Table I. Hydrogen Bonding in 17

D–H···A	symmetry of A relative to D	d(D···A), Å	d(H···A), Å	∠(D–H··A), deg
N9–H9A···O8	$x+1, y+1, z$	2.792 (4)	2.11	132
N9–H9B···O2′	$x, y+1, z$	3.135 (4)	2.42	152
N6–H6···OW	$x, y+1, z-1$	2.829 (3)	1.88	166
O2′–HO2′···O5′	$x, y-1, z$	2.671 (3)	1.96	159
O3′–HO3′···S10	$x, y-1, z+1$	3.205 (2)	2.28	174
O5′–HO5′···N4	x, y, z	2.950 (3)	2.08	162
OW–HWA···O3′	$x+1, y, z$	2.858 (3)	2.02	172
OW–HWB···S10	$x, y, z+1$	3.569 (2)	2.75	158

thioguanosine analogue **17**, was used for single-crystal X-ray diffraction analysis. The molecular conformation and atom labeling are shown in Figure 1. The molecule assumes the syn conformation, which is stabilized by the intramolecular O5′–HO5′··N4 hydrogen bond (see Table I). The glycosidic torsion angle (O4′–C1′–N3–C2) is −120.0 (2)° and the glycosidic bond is 1.458 (3) Å. These features have been observed in 8-substituted guanosines, e.g. 8-bromo-,[23] 8-chloro-,[24] and 7-methyl-8-oxoguanosines.[25] The thiazolopyrimidine ring is nearly planar [C7 deviates greatest, by 0.031 (2) Å]. The substituents S10 and C1′ deviate from the plane by 0.1604 (5) and 0.163 (2) Å, respectively. The dihedral angle between the thiazole and pyrimidine planes is 1.36 (6)°, a value similar to the aforementioned guanosines.[23-25] The S1–C2 and S1–C7A bond lengths are 1.764 (3) and 1.737 (3) Å, respectively. These large bonds enlarge the five-membered ring to a size that is comparable to a six-membered ring.

The ribose has the $C_{2'}$ endo–$C_{1'}$ exo ($^2T_1$) conformation with a pseudorotation angle of 150°.[26] The side chain is gauche$^-$–gauche$^+$ with torsion angles about the C4′–C5′ bond of −64.6 (2)° and 53.7 (2)° for O4′ and C3′, respectively. Bond lengths and bond angles are in the normal ranges. All nitrogen and oxygen (including the water solvent) protons participate in hydrogen bonding (Table I). The thioxo group S10 is a proton acceptor whereas the ring sulfur S1 is not.

The results of these crystallographic studies confirm the structural assignment of **17** as having the β-configuration with the site of glycosylation at N3, and consequently, these assignments apply to its precursor, **6**, and all nucleosides derived therefrom. Since **7** is formed by simple deprotection of **6**, these assignments hold for **7** as well.

Immunological Studies

All deprotected nucleosides were evaluated for their effects on several immune functions in mouse cells both in vitro and in vivo in direct comparison to 8-bromo-, 8-mercapto-, and 7-methyl-8-oxoguanosine, which were used as positive controls. Of the thiazolopyrimidines, only the guanosine analogues **7** and **14** showed significant immunopotentiation and therefore were the only compounds included in the tables for comparison. The inosine (**10**), adenosine (**32**), xanthosine (**8**) and 6-mercaptoguanosine (**17**) analogues were devoid of significant immunomodulatory activity. The results reported here reflect representative findings of many repeat experiments in each of the areas described below.

First, the ability of compounds to activate murine spleen cells to proliferate in vitro was determined by measuring new DNA synthesis in a [^{3}H]thymidine incorporation assay. The relative potency of compounds **1–3**, **7**, and **14** are compared in Table II and the results indicate that **1** was the least active while **7** was the most effective for inducing spleen cells to carry out new DNA synthesis. Under the conditions of these experiments, all compounds showed some degree of significant dose-dependent mitogenic activity. Compound **1**, at the maximum concentration tested, produced about a 3-fold increase in thymidine incorporation compared to controls whereas **7** gave approximately a 50-fold increase. Interestingly, the maximum level of incorporation produced by the two 8-oxo compounds, **3** and **7**, was about the same, but **7** appeared to be twice as potent as **3**, reaching the near maximum level at about half the concentration required for that of **3**. Similarly, the relative potencies of **2** and **14**, the two 8-mercapto compounds, showed a similar profile, albeit at a lower overall activity level. The guanosines were found to be nontoxic to cells at least up to the highest concentration tested in vitro.

Second, the ability of compounds to augment murine natural killer (NK) cell activity against T-cell lymphoma (YAC-1) target cells was determined in an ex vivo system; that is, animals were treated with the test agents and then spleen cells were removed and assayed in vitro for cytotoxicity against the target cells. The results shown in Table III indicate that all compounds manifested a significant adjuvant effect with respect to the control group ($p < 0.001$, by the two-tailed t test) at both effecter to target ratios. As in the case of the thymidine incorporation studies, compound **7** was found to be the most active, particularly at the lower effecter/target ratio (50:1). The apparent order of activity in this model, in descending order, was found to be **7 > 3 > 2 > 1 ≥ 14**. Compound **14** exhibited only moderate activity in this system.

Finally, compounds were tested in vivo for their ability to provide protection against a lethal Semliki Forest virus infection in mice. The results of this study are shown in Table IV and indicate that at doses of 100 and 200 mg per kg per day given intraperitoneally in half-daily doses for one day, compound **7** protected 92% of the mice while **14** provided about equal protection but was somewhat more toxic than **7**. The 8-substituted guanosines **1–3**, by comparison, were less active with maximum protection ranging from insignificant (in the case of **1**) to 75% (in the case of **3**). Thus, the order of bioactivity of the compounds tested in this model, in descending order, was found to be **7 ≥ 14 > 3 > 2 > 1**. It should be noted here that these compounds were devoid of significant in vitro antiviral activity against this virus, suggesting that protection was conferred by immunopotentiation rather than by direct antiviral properties. Indeed, **7** has been shown in our laboratory to induce interferon[27] production and this cytokine is likely the principal factor responsible for the in vivo antiviral effect. Extensive accounts of the antiviral and immunological studies of **7** (reported as 7-thia-8-oxoguanosine) and related compounds will be reported elsewhere.[28,29]

Conclusion

Analogues of the naturally occurring purine nucleosides adenosine, inosine, xanthosine, and guanosine in the thiazolo[4,5-*d*]pyrimidine ring system were successfully

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Table II. The Effect of Compounds on New DNA Synthesis in Spleen Cells of CBA/CaJ Mice[a]

compd	[^{3}H]thymidine incorporation in spleen cells (CPM)[b]				
	0 mM	0.01 mM	0.05 mM	0.20 mM	0.40 mM
1	5204 ± 512	4387 ± 317	4835 ± 490	9199 ± 1457[c]	16771 ± 671[c]
2	3633 ± 631	3911 ± 421	5372 ± 548[c]	23092 ± 868[c]	40690 ± 921[c]
3	2000 ± 184	6012 ± 618[c]	35980 ± 884[c]	72000 ± 1040[c]	94100 ± 1431[c]
7	2000 ± 216	12000 ± 960[c]	54150 ± 972[c]	96680 ± 1200[c]	107000 ± 1621[c]
14	3111 ± 88	4230 ± 482[c]	15711 ± 1082[c]	43779 ± 1111[c]	53493 ± 2538[c]

[a] Spleen cells were incubated with compounds for a total of 48 h. After 24 h of incubation, [^{3}H]thymidine (0.5 μCi/0.02 mL) was added. Cells were harvested and [^{3}H]thymidine incorporation was determined. [b] CPM values are expressed as the mean ± standard deviation of assays run in quadruplicate. [c] Statistically significant ($p < 0.01$), determined by the two-tailed t test.

Table III. In Vivo Effect of Guanosines on Murine NK Cell Activity[a]

compd[d]	% cytotoxicity[b]	
	50:1[c]	100:1[c]
2% bicarb[f]	18.30 ± 0.31[e]	25.95 ± 0.75
1	31.33 ± 1.92	45.44 ± 1.76
2	43.53 ± 2.52	61.33 ± 1.74
3	58.80 ± 0.12	57.89 ± 1.13
7	74.80 ± 2.11	67.80 ± 1.68
14	28.89 ± 1.70	41.38 ± 0.62

[a] CBA/CaJ mice (two per group, female, 6–8 weeks old). [b] Spleen cells were isolated, pooled, and cytotoxicity was determined against YAC-1 cells in a 4-h ^{51}Cr-release assay. Assays were performed in triplicate. [c] Effecter to target ratio. [d] Mice were injected intraperitoneally with compound (150 mg/kg) in 2% aqueous sodium bicarbonate in half-daily doses (75 mg/kg bid, one day treatment). [e] Standard deviation. [f] 2% aqueous sodium bicarbonate (0.1 mL/injection) used as placebo control.

Table IV. Effects of Guanosines against a Semliki Forest Virus Infection in Mice

compd	dose,[a] mg/kg	survivors/ total (%)	mean survival time,[b] days
placebo[c]		0/12 (0)	7.1 ± 1.6[d]
1	100	0/12 (0)	7.7 ± 1.7
	200	2/11[e] (18)	7.1 ± 2.0
2	100	6/12 (50)[f]	8.0 ± 3.5
	200	3/12 (25)	7.5 ± 1.4
3	100	6/12 (50)[f]	7.7 ± 2.0
	200	9/12 (75)[f]	6.3 ± 0.6
7	100	11/12 (92)[f]	8.0 ± 0.0
	200	11/12 (92)[f]	6.0 ± 0.0
14	100	4/9[e] (44)	7.0 ± 1.9
	200	11/11[e] (100)[f]	>21

[a] Half-daily doses were administered intraperitoneally at −24 and −18 hours relative to virus inoculation. [b] Of mice that died. Survivors lived through 21 days. [c] 2% sodium bicarbonate used as placebo control. [d] Standard deviation. [e] Where group sizes are less than 12, some mice died a day or two after administering the compound and were eliminated from the results. [f] Statistically significant ($p < 0.025$), determined by the two-tailed Fisher exact test.

synthesized as well as related nucleosides of interest such as the 8-mercapto- and 6-mercaptoguanosine analogues. The compounds were evaluated for their ability to serve as mitogens in murine spleen cells and were compared to the known mitogens 8-bromoguanosine, 8-mercaptoguanosine, and 7-methyl-8-oxoguanosine. Only the guanosine analogues showed significant activity relative to the positive control compounds. Thus, the *N*-methyl portion of **3** can be replaced by a sulfur atom, resulting in an analogue, **7**, which exhibits equal or even greater immunological activity than the parent purine nucleoside to which it is analogous. Compound **7** exhibited greater immunoactivity than any of the other guanosine analogues and derivatives in all test systems, which included murine spleen cell proliferation, ex vivo natural killer cell assay, and in vivo prophylaxis against Semliki Forest virus in mice. A major mode of action of these guanosine analogues is thought to be the induction of interferon production.

Table V. Summary of Crystal and Refinement Data for **17**

formula	$C_{10}H_{12}N_4O_5S_2 \cdot H_2O$
formula weight	350.36
crystal system	triclinic
space group	$P\bar{1}$
a, Å	5.285 (3)
b, Å	6.792 (3)
c, Å	10.692 (9)
α, deg	74.38 (5)
β, deg	84.28 (7)
γ, deg	73.09 (4)
V, Å^3	353.6 (4)
Z	1
ρ_{calcd}, g cm^{-3}	1.65
$F(000)$, electrons	182
radiation λ, Å	Mo Kα, 0.71073
crystal size, mm	0.35 × 0.20 × 0.15
μ, cm^{-1}	3.95
max 2θ, deg	60
total refltns, measd, unique	2297, 2297
observed refltns	2177
no. of variables	310
S (goodness of fit)	1.12
R, wR	0.028, 0.035
max Δ/σ	0.022
max, min in ΔF map	0.30, −0.19

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus or on a Haake-Buchler digital melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra (b = broad singlet) were determined at 300.1 MHz with an IBM NR300AF spectrometer. The chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane as internal standard. Ultraviolet spectra (UV: sh = shoulder) were recorded on a Beckman DU-50 spectrophotometer. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. Evaporations were carried out under reduced pressure with the bath temperature below 40 °C. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM reagents). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. HPLC purity determinations were done with a Waters 600 solvent delivery system equipped with a Waters 990 photodiode array detector and a Beckman ultrasphere 5-μm reversed-phase column (4.6 × 250 mm).

X-ray Crystallography. Crystal and intensity data were measured on a Nicolet R3 automated diffractometer utilizing Mo Kα radiation. Relevant data are found in Table V. A variable speed θ–2θ scan procedure was used for data collection. A trial structure containing all non-hydrogen atoms (including the solvent oxygen) was obtained from an E map using direct methods. These atoms were refined anisotropically. Hydrogens bonded to carbons in the sugar were placed at ideal positions (C–H distance of 0.96 Å) while all other hydrogens were located in difference maps and allowed to ride on their nearest neighbors; however, hydrogen isotropic thermal parameters were refined. The final R values are listed in Table V. The structure was refined by least squares, minimizing the function $\sum w(|F_o| - |F_c|)^2$ where $w = (\sigma_F^2 + 0.00059F^2)^{-1}$. Solution and refinement programs used in the investigation are contained in the SHELXTL[30] package. Figure 1

was produced with ORTEPII.[31] Scattering factors were obtained from the *International Tables for X-ray Crystallography*.[32]

5-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (6). A mixture of dry 5-aminothiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione[13] (4; 5.5 g, 30 mmol), hexamethyldisilazane (HMDS, 100 mL), ammonium sulfate (15 mg), and pyridine (10 mL) was heated under reflux for 4 h with the exclusion of moisture. Excess HMDS was removed by distillation to provide the syrupy bis-silyl derivative. The bis-silyl intermediate was dissolved in dry acetonitrile (300 mL) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**5**; 15.1 g, 30 mmol) was added followed by trimethylsilyl trifluoromethanesulfonate (9.3 mL, 42 mmol). The clear reaction mixture was stirred at ambient temperature for 16 h. The solvent was evaporated to dryness and the residual syrup was dissolved in EtOAc (600 mL). The solution was washed with 5% $NaHCO_3$ solution (2 × 150 mL), and the dried (Na_2SO_4) organic layer was evaporated. The residual syrup was triturated with ether to yield 18.1 g (96%). The resulting foam was purified on a silica gel column by flash column chromatography using $CHCl_3$–MeOH (9:1, v/v) as the solvent. Recrystallization of the residue from EtOH gave 5-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**6**) as colorless crystals: yield 14.5 g, 77%; mp 248–250 °C; UV λ_{max} (pH 1, 7) 219 nm (ε 28000), 224 sh (27600), 301 (8500); UV λ_{max} (pH 11) 218 nm (ε 27800), 273 (6900). Anal. ($C_{31}H_{24}N_4O_9S$) C, H, N, S.

5-Amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (7). To a suspension of **6** (5.0 g, 8.0 mmol) in methanol (100 mL) was added $NaOCH_3$ (2.10 g, 40.0 mmol) and the mixture, which slowly became clear, was stirred at room temperature for 16 h. The reaction mixture was neutralized with Dowex-50 H^+ resin and filtered, and the filtrate was evaporated to dryness. The residue was triturated with ether (2 × 75 mL) and the ether-insoluble solid was crystallized from water (80 mL, charcoal) to give a white powder: yield 2.0 g, 78%; mp 238 °C dec; UV λ_{max} (pH 1) 215 nm (ε 22800), 245 (6900), 301 (8400); UV λ_{max} (pH 7) 215 nm (ε 22100), 245 (6900), 301 (8000); UV λ_{max} (pH 11) 245 nm (ε 5700), 291 (6000); ^1H NMR (DMSO-d_6) δ 5.79 (d, J = 5.32 Hz, 1 H, $C_1'H$), 6.90 (s, 2 H, NH_2), 11.12 (s, 1 H, NH), and other sugar protons. Anal. ($C_{10}H_{12}N_4O_6S\cdot H_2O$) C, H, N, S.

3-β-D-Ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,5,7-(3*H*,4*H*,6*H*)-trione (8). To a suspension of **7** (0.76 g, 2.4 mmol) in glacial acetic acid (150 mL) was added dropwise a solution of sodium nitrite (1.5 g, 21.7 mmol) in water (15 mL) with stirring. After 30 min the suspension became clear and the stirring was continued at room temperature overnight. The white solid which had separated was filtered, washed with cold water, and dried. Recrystallization from hot water gave fine, colorless crystals of **8**: yield 0.3 g, 40%; mp 250 °C dec; UV λ_{max} (pH 1) 293 nm (ε 5500); UV λ_{max} (pH 7) 212 nm (ε 14200), 301 (6100); UV λ_{max} (pH 11) 204 nm (ε 21900), 301 (5600); ^1H NMR (DMSO-d_6) δ 5.83 (d, J = 7.8 Hz, 1 H, $C_1'H$), 11.5 (s, 1 H, N*H*), 12.0 (b, 1 H, NH), and other sugar protons. Anal. ($C_{10}H_{11}N_3O_7S$) C, H, N, S.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (9). To a solution of **6** (6.65 g, 10.6 mmol) in dry THF (350 mL) was added *tert*-butyl nitrite (6.2 mL, 52.3 mmol) and the mixture was stirred at room temperature for 1 h. Additional nitrite reagent (2.0 mL) was added and the mixture was stirred at 50–60 °C overnight. The mixture was evaporated and the residue was purified by flash column chromatography on silica gel using 8–10% acetone in CH_2Cl_2 followed by 10–11% acetone in CH_2Cl_2. The desired product eluted last to yield 3.45 g (46%) of **9** as a foam: UV λ_{max} (EtOH) 220 nm (ε 46600), 259 sh (11000), 271 sh (8400); ^1H NMR (DMSO-d_6) δ 6.31 (d, J = 6.45 Hz, 1 H, $C_1'H$), 7.38–7.98 (m, 15 H, benzoyl aromatics), 8.25 (s, 1 H, C_5H), 13.16 (b, 1 H, N_6H, exchanged with D_2O), and other sugar protons. Anal. ($C_{31}H_{23}N_3O_9S$) C, H, N, S.

3-β-D-Ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7-(3*H*,6*H*)-dione (10). Compound **9** (1.0 g, 1.63 mmol) was combined with methanolic ammonia (saturated at 0 °C, 50 mL) and heated at 90 °C for 14 h in a steel bomb. The solvent was evaporated and the residue was treated with hot benzene, which was decanted off. The resulting solid was purified by silica gel flash chromatography using chloroform and then $CHCl_3$–MeOH (6:1) to yield 280 mg (57%) of **10** after crystallization from water: mp 216–218 °C; UV λ_{max} (pH 1) 217 nm (ε 25300), 259 (9700), 286 (6300); UV λ_{max} (pH 7, 11) 260 nm (ε 10500); ^1H NMR (DMSO-d_6) δ 5.85 (d, J = 5.1 Hz, 1 H, $C_1'H$), 8.30 (s, 1 H, C_5H), 13.09 (b, 1 H, N_6H, exchanges with D_2O), and other sugar protons. Anal. ($C_{10}H_{11}N_3O_6S$) C, H, N, S.

5-Amino-2,3-dihydro-2-thioxothiazolo[4,5-*d*]pyrimidin-7-(6*H*)-one (12). A suspension of 5-amino-2-chlorothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one[13] (11; 1.5 g, 7.4 mmol) in ethylene glycol (30 mL) was heated to 110 °C and $NaSH\cdot xH_2O$ (420 mg, 74 mmol) was added. A clear solution was not obtained, however, until an additional 250 mg were added. The clear solution was stirred at 110 °C for 2 h and then the reaction mixture was cooled to room temperature and poured into ice (300 mL), and the pH was adjusted to 2–3 with 10% HCl. The resulting pink gelatinous mixture was boiled for 1 h and the pink solid was collected by filtration through a medium-frit glass filter, washed with water, and dried: yield 1.2 g, 81%; an analytical sample was prepared by flash column chromatography using EtOAc–MeOH–H_2O–acetone (7:1:1:1); mp > 300 °C; UV λ_{max} (pH 1) 243 nm (ε 13700), 266 (16500), 351 (17200); UV λ_{max} (pH 7) 262 nm (ε 14800), 345 (12700); UV λ_{max} (pH 11) 250 nm (ε 19300), 335 (14300); ^1H NMR (DMSO-d_6) δ 6.91 (s, 2 H, NH_2), 11.18 (s, 1 H, N_6H), 13.78 (s, 1 H, N_3H). Anal. ($C_5H_4N_4OS_2$) C, H, N, S.

5-Amino-2,3-dihydro-2-thioxo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (13). Compound **12** (1.0 g, 5 mmol) was glycosylated in the same manner as that used to prepare **6**, requiring HMDS (20 mL), benzoyl-blocked sugar (**5**; 2.52 g, 5 mmol), and TMS-triflate (1.45 mL, 7.5 mmol). At the end of the 16-h reaction period, the reaction mixture was heated at 70 °C for 3 h in order to rearrange any putative *S*-glycoside formed to the more stable *N*-glycoside. After the same workup as used in **6**, **13** (2.1 g crude) was purified by flash column chromatography using hexanes–acetone (1:1) and crystallized from toluene–EtOAc: yield 1.9 g, 59%; mp 230–233 °C (darkens at >195 °C). Anal. ($C_{31}H_{24}N_4O_8S_2$) C, H, N, S.

5-Amino-2,3-dihydro-2-thioxo-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (14). To a solution of **13** (1.25 g, 1.94 mmol) in dry methanol (100 mL) was added sodium methoxide powder until the pH reached 10. The solution was stirred overnight and then neutralized with Dowex-50 H^+ resin and filtered. After evaporation of the filtrate, the residue was washed with ether to remove methyl benzoate and the crude material was crystallized from water: yield 520 mg, 81%; mp >220 °C dec; UV λ_{max} (pH 1) 221 nm (ε 20700), 245 (21000), 266 (21200), 290 (12500), 352 (25700); UV λ_{max} (pH 7) 244 nm (ε 26100), 266 (25600), 353 (30400); UV λ_{max} (pH 11) 243 nm (ε 24500), 265 (22400), 355 (26800); ^1H NMR (DMSO-d_6) δ 6.48 (d, J = 3.00 Hz, 1 H, $C_1'H$), 6.99 (s, 2 H, NH_2), 11.47 (s, 1 H, NH), and other sugar protons. Anal. ($C_{10}H_{12}N_4O_5S_2\cdot H_2O$) C, H, N, S.

5-[[(*N*,*N*-Dimethylamino)methylene]amino]-7-chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (15). Dry purified **6** (10 g, 16 mmol) was dissolved in dry methylene chloride (350 mL) and a solution of freshly distilled thionyl chloride (40 mL) and dry DMF (20 mL) in dry methylene chloride was added dropwise over a 2-h period and the reaction was kept at 60 °C (reflux) for 16 h. The reaction mixture was poured carefully into an ice-cold $NaHCO_3$ solution and stirred for 30 min. The layers were separated, the aqueous layer was extracted (2 × 150 mL) with methylene chloride, and the combined layers were dried over Na_2SO_4 and evaporated in vacuo. The residual syrup was purified by passing it thorugh a silica gel column (4 × 40 cm) and eluting with $CHCl_3$–acetone (4:1), to obtain the chloro compound as a white foam: 8.6 g, 84%, mp 88–90 °C. Anal. ($C_{34}H_{28}ClN_5O_8S$) C, H, Cl, N, S.

5-Amino-6,7-dihydro-7-thioxo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (16).

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(32) *International Tables for X-Ray Crystallography*; Ibers, J. A., Hamilton, W. C., Eds.; Kynoch Press: Birmingham, England, 1974; Vol. 4, p 99.

Method 1. A mixture of **15** (3.3 g, 5 mmol), thiourea (0.719 g, 1 mmol), and EtOH (100 mL) was heated under reflux for 6 days. The reaction mixture was evaporated, and the residue was extracted with $CHCl_3$ (200 mL). The solvent was evaporated to dryness in vacuo, and the residue was purified by silica gel column chromatography with $CHCl_3$–acetone (7:1) as the eluent. After evaporation, the residue was crystallized from EtOH to afford a colorless powder: yield 1.9 g, 58%; mp 227–229 °C; UV λ_{max} (pH 1, 7) 234 nm (ϵ 26000), 280 sh (9000), 365 (11800); UV λ_{max} (pH 11) 230 nm (ϵ 40500), 267 (8700), 327 (14100). Anal. ($C_{31}H_{24}N_4O_8S_2$) C, H, N, S.

Method 2. To a solution of **6** (1 g, 1.6 mmol) in pyridine (50 mL) was added with stirring P_2S_5 (1.5 g, 6.2 mmol). The solution was refluxed gently (bath temperature 130–140 °C) for 29 h. The reaction mixture was evaporated to dryness in vacuo. The excess P_2S_5 was decomposed by the addition of H_2O (200 mL) at 60 °C. The mixture was stirred for 1 h and then left at room temperature overnight. The resulting solid was filtered, dissolved in $CHCl_3$, and dried (Na_2SO_4) and the solvent was removed under vacuum. The residue was purified by silica gel column chromatography with $CHCl_3$–acetone 7:1 as the eluent. After concentration, the residue was crystallized from EtOH to give **16** (0.43 g, 43%). The physicochemical properties of compound **16** prepared by method 2 were found to be identical in all respects with those of the nucleoside prepared by method 1 above.

5-Amino-6,7-dihydro-7-thioxo-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (17). Method 1. A solution of **16** (1.0 g, 1.6 mmol) in methanol (50 mL) was adjusted to pH 9 with $NaOCH_3$ and stirred at room temperature for 16 h. The reaction mixture was evaporated to dryness and the residue was triturated with ether (2 × 75 mL). The filtered, ether-insoluble solid was dissolved in water (15 mL) and the solution was acidified with acetic acid whereupon the crude product precipitated. Recrystallization of this material from EtOH–H_2O gave colorless prisms: yield 0.47 g, 87%; mp 185–187 °C; UV λ_{max} (pH 1) 214 nm (ϵ 27000), 230 sh (14000), 263 (6700), 354 (21600); UV λ_{max} (pH 7) 213 nm (ϵ 25900), 247 (9100), 266 sh (7700), 334 (12100), 353 (11800); UV λ_{max} (pH 11) 247 nm (ϵ 12300), 266 sh (8800), 327 (16100); ^{1}H NMR (DMSO-d_6) δ 5.76 (d, J = 5.32 Hz, 1 H, C_1'H), 7.22 (s, 2 H, NH_2), 12.41 (s, 1 H, NH), and other sugar protons. Anal. ($C_{10}H_{12}N_4O_5S_2 \cdot H_2O$) C, H, N, S.

Method 2. A solution of **16** (1.0 g, 1.6 mmol) in methanolic ammonia (saturated at 0 °C, 60 mL) was stirred at room temperature for 48 h. The solvent was evaporated to dryness and the residue was triturated with boiling benzene (2 × 100 mL). The benzene-insoluble solid was crystallized from EtOH–H_2O to give **17** (0.36 g, 67%). The compound prepared by this method was identical with compound **17** prepared by method 1 above, as judged by spectral and physical data.

5-Amino-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)tetrazolo[5,1-*f*]thiazolo[4,5-*d*]pyrimidin-8(7*H*)-one (18). To a solution of **15** (3.0 g, 4.6 mmol) in dry DMF (30 mL) was added sodium azide (0.3 g, 4.6 mmol) and the mixture was stirred at room temperature for 3 days. After evaporation of the solvent, the residue was dissolved in EtOAc (250 mL), washed with water (2 × 50 mL), dried over sodium sulfate, and evaporated. The resulting foam was purified by silica gel column chromatography using $CHCl_3$–acetone 7:1. The product was crystallized from EtOH to give a white powder: yield, 2.0 g, 67%; mp 112–114 °C; IR showed no azide band in the region of 2100–2200 cm^{-1}; UV λ_{max} (pH 1) 232 nm (ϵ 39800), 270 (13500), 307 (11100), 353 (6500); UV λ_{max} (pH 7, 11) 234 nm (ϵ 22200), 268 (9700), 306 (7100), 353 (9500); UV λ_{max} (EtOH) 211 nm (ϵ 28000), 230 (52500), 264 (14400), 299 (17300). Anal. ($C_{31}H_{23}N_7O_8S$) C, H, N, S.

2,5,7-Trichlorothiazolo[4,5-*d*]pyrimidine (20). A mixture of 2-chlorothiazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione[13] (**19**; 15.8 g, 78 mmol), $POCl_3$ (220 mL), and *N*,*N*-dimethylaniline (12.3 g, 0.1 mol) was refluxed for 3 h. The excess $POCl_3$ was removed under reduced pressure and the residue was poured into ice–water (500 mL) with stirring. The resulting aqueous solution was extracted with $CHCl_3$ (3 × 400 mL) and the organic layer was washed with water (2 × 400 mL), 0.1 N NaOH (2 × 300 mL), and water (2 × 400 mL) successively and then dried over Na_2SO_4. Evaporation of the chloroform produced a residue which was purified by silica gel column chromatography using $CHCl_3$ to provide the title compound (**20**) after crystallization from EtOH: yield 13.8 g, 74%; mp 121–122 °C; UV λ_{max} (pH 1, 7, 11) 296 nm (ϵ 10800). Anal. ($C_5Cl_3N_3S$) C, Cl, N.

5,7-Dichloro-2-(*N*-methylanilino)thiazolo[4,5-*d*]pyrimidine (21). This compound was isolated as a minor product from the column purification described in **20** above: yield, 0.2 g, 1%; mp 176–177 °C (EtOH). Anal. ($C_{12}H_8N_4Cl_2S$) C, H, Cl, N. The structural assignment was verified by single-crystal X-ray analysis and is detailed elsewhere.[15]

5,7-Dichlorothiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (22). A suspension of the trichloro compound (**20**; 3.0 g, 12 mmol) in 1 N NaOH (35 mL) was heated at 60 °C for 1 h. The solution was treated with decolorizing carbon and then acidified with 10% aqueous HCl. The resulting precipitate was collected and reprecipitated from dilute base with glacial acetic acid to provide **22** as orange needles (1.38 g, 50%): mp 191–192 °C; UV λ_{max} (pH 1) 254 nm (ϵ 5300), 290 (11400); UV λ_{max} (pH 7, 11) 226 nm (ϵ 28900), 300 (14100). Anal. ($C_5HCl_2N_3OS$) C, H, Cl, N. Single crystal X-ray analysis of **22** showed the structural assignment to be correct.[16]

5,7-Dichloro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (24). A finely powdered mixture of **22** (3.7 g, 16 mmol), 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (**23**; 5.3 g, 16 mmol), and bis(*p*-nitrophenyl) phosphate (20 mg) was heated at 170 °C for 10 min under reduced pressure. After cooling of the reaction mixture to room temperature, the brown, solid mass was dissolved in EtOAc (500 mL) and washed with saturated aqueous sodium bicarbonate (3 × 300 mL). The dried (Na_2SO_4) organic layer was evaporated to yield a syrup, which was purified by silica gel column chromatography (4 × 40 cm) using toluene–EtOAc (5:1). The resulting syrup was crystallized from ethanol to give a white powder: yield 6.4 g, 80%; mp 125–126 °C; ^{1}H NMR (DMSO-d_6) δ 1.99, 2.06, 2.08 (3 s, 9 H, acetyls), 6.07 (d, J = 3.40 Hz, 1 H, C_1'H), and other sugar protons. Anal. ($C_{16}H_{15}Cl_2N_3O_8S$) C, H, Cl, N, S.

7-Amino-2-chlorothiazolo[4,5-*d*]pyrimidine (26). To a suspension of 2,7-diaminothiazolo[4,5-*d*]pyrimidine[13] (**25**; 16.3 g, 97.3 mmol) in water (200 mL) at 55 °C was added enough 1 *N* NaOH (about 100 mL) to dissolve the starting material and sodium nitrite (8.0 g) was then added. This solution was then added dropwise over 30 min to a solution containing concentrated aqueous HCl (400 mL), water (100 mL), and LiCl (60 g) at 30 °C. The resulting mixture was warmed to 45 °C for 15 min and then hot water (1 L, 90 °C) was added. The reaction mixture was stirred overnight at room temperature and filtered to remove unreacted starting material, and the filtrate was neutralized with solid NaOH to pH 4. The resulting solid was filtered off, washed with water, and dried to yield **26**: 5.38 g (34%); recrystallization from water gave an analytical sample; mp >234 °C dec; UV λ_{max} (pH 1) 220 nm (ϵ 22400), 266 (8600), 290 (8300); UV λ_{max} (pH 7, 11) 232 nm (ϵ 33800), 286 (9700); ^{1}H NMR (DMSO-d_6) δ 7.82 (b, 2 H, NH_2, exchanges with D_2O), 8.41 (s, 1 H, C_5H). Anal. ($C_5H_3ClN_4S$) C, H, Cl, N, S.

7-Aminothiazolo[4,5-*d*]pyrimidine-2(3*H*)-thione (27). A suspension of compound **26** (1.11 g, 5.9 mmol) in dry DMF (10 mL) was cooled in an ice bath to 0 °C and $NaSH \cdot xH_2O$ (0.87 g, 11.8 mmol) was added. The resulting clear solution was stirred overnight at 0 °C and then at room temperature for 2 h. The reaction mixture was poured into ice (300 mL) and the pH was adjusted to 3–4 with glacial acetic acid. The solid precipitate was filtered, washed with water, and dried to yield 0.96 g (88%). An analytical sample was prepared by crystallization from DMF–water: mp >370 °C; UV λ_{max} (pH 1) 224 nm (ϵ 15000), 248 (16900), 263 (13500), 345 (28700); UV λ_{max} (pH 7, 11) 228 nm (ϵ 18900), 259 (23400), 329 (23800); ^{1}H NMR (DMSO-d_6) δ 7.57 (b, 2 H, NH_2, exchanges with D_2O), 8.23 (s, 1 H, C_5H), 14.13 (b, 1 H, N_3H, exchanges with D_2O). Anal. ($C_5H_4N_4S_2$) C, H, N, S.

7-Aminothiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (28). To a suspension of **27** (770 mg, 4.2 mmol) in water (30 mL) was added 1 N NaOH (4.2 mL) and 30% H_2O_2 (1.0 mL). The mixture was stirred for 1 h at room temperature. Additional peroxide (2.0 mL) and hydroxide (5.0 mL) were added and the mixture was stirred for 1 h at 70 °C. The reaction mixture was filtered and the filtrate was neutralized with glacial acetic acid. The resulting precipitate was filtered while still hot, washed with cold water, and dried to yield 0.52 g (74%); mp >370 °C; UV λ_{max} (pH 1) 220 nm (ϵ 20400), 267 (7600), 290 (6900); UV λ_{max} (pH 7, 11) 231 nm (ϵ 24200), 285

(8100); ^{1}H NMR (DMSO-d_6) δ 7.18 (b, 2 H, NH_2, exchanges with D_2O), 8.12 (s, 1 H, C_5H), 12.30 (b, 1 H, N_3H, exchanges with D_2O). Anal. ($C_5H_4N_4OS$) C, H, N, S.

7-Amino-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2-one (29). Compound **28** (460 mg, 2.7 mmol) was glycosylated in the same manner as that used to prepare **6**, requiring HMDS (30 mL), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**5**; 1.5 g, 3.0 mmol), and TMS-triflate (0.76 mL, 3.9 mmol). The reaction mixture was allowed to stir overnight at room temperature and was then worked up as described for **6** to yield 1.6 g (95%) of **29** isolated as a foam: UV λ_{max} (pH 1) 236 nm (ε 40 900), 320 (18 100); UV λ_{max} (pH 7, 11) 236 nm (ε 41 500), 318 (18 500); ^{1}H NMR (DMSO-d_6) δ 6.45 (d, J = 2.73 Hz, 1 H, $C_{1'}H$), 7.4–8.0 (m, 17 H, benzoyl aromatics and NH_2), 8.59 (s, 1 H, C_5H), and other sugar protons. Anal. ($C_{31}H_{24}N_4O_8S$) C, H, N, S.

7-Amino-4-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2-one (30). Compound **29** (310 mg, 0.51 mmol) was dissolved in dry methanol (35 mL) and cooled to 5 °C. To this solution was added solid sodium methoxide (82 mg, 1.5 mmol) and the solution was stirred at room temperature for 5 h. The mixture was neutralized with Dowex-50 H^+ resin, filtered, and evaporated to dryness. The residue was triturated with ethyl ether and then recrystallized from aqueous ethanol to yield colorless needles: 120 mg, 80%; mp 132–134 °C; UV λ_{max} (pH 1) 227 nm (ε 17 230), 301 (15 750); UV λ_{max} (pH 7, 11) 233 nm (ε 22 300), 305 (19 100); ^{1}H NMR (DMSO-d_6) δ 5.96 (d, J = 3.51 Hz, 1 H, $C_{1'}H$), 7.75 (b, 2 H, NH_2, exchanges with D_2O), 8.77 (s, 1 H, C_5H), and other sugar protons. Anal. ($C_{10}H_{12}N_4O_5S \cdot 0.2H_2O$) C, H, N, S.

7-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (31). Compound **28** (1.22 g, 7.25 mmol) was glycosylated as described for the preparation of **6**, requiring HMDS (35 mL), benzoyl-blocked sugar (**5**; 4.4 g, 8.7 mmol), and TMS-triflate (2.0 mL, 10.3 mmol). After stirring overnight at room temperature, the reaction mixture was refluxed for 2 days and then worked up in the usual manner. The crude mixture was subjected to flash silica gel column chromatography using a gradient of methylene chloride to methylene chloride–acetone 10:1 (v/v) and yielded two products. The first to elute from the column was the minor product, which amounted to 660 mg. The second and major product off the column was obtained as a foam and assigned as the desired 3-ribosyl isomer by UV, ^{1}H NMR, and X-ray diffraction: yield 1.04 g, 24%; UV λ_{max} (EtOH) 232 nm (ε 59 400), 285 (27 600); ^{1}H NMR (DMSO-d_6) δ 6.34 (t, 1 H, $C_{1'}H$), 7.39–7.98 (m, 17 H, benzoyl aromatics and NH_2), 8.19 (s, 1 H, C_5H), and other sugar protons. Anal. ($C_{31}H_{24}N_4O_8S$) C, H, N, S.

7-Amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2-(3*H*)-one (32). Compound **31** (0.76 g, 1.2 mmol) was deblocked in the same manner as described for **29** above using sodium methoxide (200 mg, 3.7 mmol) in dry MeOH (50 mL). The title compound (**32**) was obtained (0.12 g, 32%) after crystallization from water: mp 248–250 °C; UV λ_{max} (pH 1) 222 nm (ε 35 100), 265 (14 300), 290 (11 400); UV λ_{max} (pH 7, 11) 215 nm (ε 45 000), 262 (13 200); ^{1}H NMR (DMSO-d_6) δ 5.91 (d, J = 5.43 Hz, 1 H, $C_{1'}H$), 7.44 (b, 2 H, NH_2, exchanges with D_2O), 8.22 (s, 1 H, C_5H), and other sugar protons. Single-crystal X-ray analysis of **32** confirms the structural assignment as the N3 β isomer.[22] Anal. ($C_{10}H_{12}N_4O_5S$) C, H, N, S.

Immunomodulation Studies. For the in vitro studies, test compounds were dissolved in RPMI medium with warming and diluted to the appropriate concentration with medium. Tritiated thymidine incorporation studies were performed as described by Goodman and Weigle.[7] Briefly, spleen cells (1×10^5/0.2 mL) from CBA/CaJ mice were incubated with various concentrations of the test compounds for 24 h in microculture plates. [^{3}H]thymidine was then added to each well and incubation was continued for an additional 24 h. Cells were harvested and tritium labeled thymidine uptake was measured in a β-counter. The optimum doses and incubation times required for maximum proliferation were selected on the basis of preliminary kinetics of activation and dose–response studies (data not shown).

To determine the effect of test compounds on NK cell cytotoxicity, mice were treated ip with drug (150 mg/kg) dissolved in 2% sodium bicarbonate (or 2% sodium bicarbonate alone as placebo) in groups of two each. After 24 h, mice were sacrificed and spleen cells from each group were pooled and assayed in triplicate at two effecter to target cell ratios for cytotoxicity against the T-cell lymphoma target YAC-1 in a standard 4-h ^{51}Cr-release experiment as described by Welsh.[33] Optimum doses of test compounds and incubation times used here were selected on the basis of preliminary experiments (reported elsewhere[28]) and were found to be the same for all the guanosines.

Semliki Forest Virus Model. Swiss Webster female mice (Charles River Labs, Wilmington, MA) weighing about 20 g each at the beginning of the experiment were inoculated intraperitoneally with test compounds (or placebo) in aqueous 2% sodium bicarbonate solution at −24 and −18 h relative to virus inoculation. The optimal dose of compound **7** (the most active compound) was established in preliminary experiments and all other guanosines were compared to **7** at these doses for relative potency. The dosing schedule indicated here was also found to be optimum for all guanosines tested. A lethal dose ($10 \times LD_{50}$) of the Semliki Forest virus (original strain) was administered by ip injection to groups of 12 mice. In a few cases, however, some mice died within a day or two of drug administration and were eliminated from the final results, as indicated in Table IV (see 1 and 14).

Registry No. 4, 30161-97-8; 4 bis-silyl derivative, 122970-61-0; **5**, 14215-97-5; **6**, 122970-39-2; **7**, 122970-40-5; **8**, 122970-41-6; **9**, 122970-42-7; **10**, 122970-43-8; 11, 30161-95-6; **12**, 122970-44-9; **13**, 122970-45-0; 14, 122970-46-1; **15**, 123002-32-4; **16**, 122970-47-2; **17**, 122970-48-3; **18**, 122970-49-4; **19**, 30161-92-3; **20**, 122970-50-7; **21**, 122970-51-8; **22**, 122970-52-9; **23**, 28708-32-9; **24**, 122970-53-0; **25**, 30162-02-8; **26**, 122970-54-1; **27**, 122970-55-2; **28**, 122970-56-3; **29**, 122970-57-4; **30**, 122970-58-5; **31**, 122970-59-6; **32**, 122970-60-9.

Supplementary Material Available: Tables of atomic positional parameters, thermal parameters, and bond lengths and angles for **17** (4 pages). Ordering information is given on any current masthead page.

(33) Welsh, R. M. *J. Exp. Med.* **1978**, *148*, 163.