116, 118695-93-5; 117, 118695-88-8; 117·$C_4H_4O_4$, 118697-01-1; 118, 118697-69-1; 119, 118697-61-3; 120, 118696-79-0; 121, 118698-21-8; 122, 118695-95-7; 123, 118697-66-8; 124, 118720-68-6; 125, 118698-51-4; 126, 135429-03-7; 127, 118698-78-5; 128, 118695-94-6; 129, 118697-54-4; 130, 118698-39-8; 131, 118698-38-7; 131·2HCl, 118698-58-1; 132, 118697-51-1; 133, 118697-13-5; 134, 118698-67-2; 135, 135429-04-8; 136, 118698-10-5; 137, 118698-09-2; 138, 135429-05-9; 139, 118697-05-5; 140, 118697-64-6; 141, 118697-62-4; 142, 118697-09-9; 143, 135429-06-0; 144, 135429-07-1; 145, 135429-08-2; 146, 135429-09-3; 147, 135429-10-6; 148, 118697-12-4; 149, 135429-11-7; 150, 135429-12-8; 151, 135429-13-9; 152, 135429-14-0; 153, 118697-63-5; 153·HCl, 118698-60-5; 154, 118696-15-4; 155, 118695-78-6; 156, 135429-15-1; 156·HCl, 135429-16-2; 157, 90887-26-6; 158, 118698-96-7; 159, 118698-99-0; (*S*)-$H_2NCH(CH_3)COOEt$·HCl, 1115-59-9; (*R*)-$H_2NCH(CH_3)$-COOMe·HCl, 14316-06-4; $H_2N(CH_2)_2COOEt$·HCl, 4244-84-2; PhCOCl, 98-88-4; *m*-ClC_6H_4COCl, 618-46-2; *p*-ClC_6H_4COCl, 122-01-0; *p*-BrC_6H_4COCl, 586-75-4; *p*-$MeOC_6H_4COCl$, 100-07-2; *p*-FC_6H_4COCl, 403-43-0; *p*-$O_2NC_6H_4COCl$, 122-04-3; *p*-$F_3CC_6H_4COCl$, 329-15-7; *p*-MeC_6H_4COCl, 874-60-2; 2,4-$Cl_2C_6H_3COCl$, 89-75-8; 5-Cl-2-C_5H_4NCOCl, 128073-03-0; 2-C_4H_3OCOCl, 527-69-5; 5-Br-2-C_4H_2OCOCl, 26726-16-9; 5-Br-2-C_4H_2SCOCl, 31555-60-9; 3-C_4H_3SCOCl, 41507-35-1; 3-C_4H_3OCOCl, 26214-65-3; *p*-$ClC_6H_4CH_2COCl$, 25026-34-0; EtNHEt, 109-89-7; PrNHPr, 142-84-7; *i*-PrNHPr-*i*, 108-18-9; BuNHBu, 111-92-2; MeNHPr, 627-35-0; H_2NCH_2COOEt, 459-73-4; $H_2N(CH_2)_3COOEt$, 5959-36-4; $H_2N(CH_2)_2NMe_2$, 108-00-9; $MeNH(CH_2)_2NMe_2$, 142-25-6; $H_2N(CH_2)_2NHCOCH_3$, 1001-53-2; $H_2N(CH_2)_3NMe_2$, 109-55-7; 2-chloro-3-nitropyridine, 5470-18-8; 2,6-dichloro-3-nitropyridine, 16013-85-7; glycinamide hydrochloride, 1668-10-6; glycine ethyl ester hydrochloride, 623-33-6; 2-pyridinecarbonyl chloride, 29745-44-6; 3-amino-2-[(2-amino-2-oxoethyl)amino]pyridine, 118699-05-1; 1-methylpiperazine, 109-01-3; pyrrolidine, 123-75-1; piperidine, 110-89-4; morpholine, 110-91-8; 1-acetylpiperazine, 13889-98-0; *N*-carbethoxypiperazine, 120-43-4; 2-aminopyridine, 504-29-0; 3-aminopyridine, 462-08-8; 4-aminopyridine, 504-24-5; 2-(4-chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine, 952-13-6; 2-chloroacetamide, 79-07-2; 2-hydroxy-6-methylpyridine-3-carboxylic acid, 38116-61-9; 2-chloro-6-methyl-3-pyridinecarboxamide, 54957-84-5; 3-amino-2-chloro-6-methyl-3-pyridinamine, 39745-40-9; 2-(4-chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine, 952-13-6; 4-chloro-3-nitropyridine, 13091-23-1.

Synthesis and Antiviral Activity of Certain Guanosine Analogues in the Thiazolo[4,5-*d*]pyrimidine Ring System

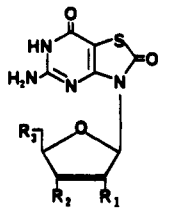
Ganesh D. Kini, Jack D. Anderson,† Yogesh S. Sanghvi,‡ Arthur F. Lewis, Donald F. Smee,§ Ganapathi R. Revankar,∥ Roland K. Robins,⊥ and Howard B. Cottam*,⊥

ICN Nucleic Acid Research Institute, 3300 Hyland Avenue, Costa Mesa, California 92626. *Received April 8, 1991*

Several sugar-modified nucleoside derivatives of the purine analogue 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]-pyrimidine-2,7-dione (1) were synthesized. Phosphorylation of 1 using $POCl_3$ resulted in 5'-monophosphate 2, which was subsequently converted to 3',5'-cyclic phosphate 3, by reported methods. 5'-Sulfamoyl derivative 4 was synthesized by treatment of the 2,3-*O*-isopropylidene derivative of 1 with chlorosulfonamide followed by acid deprotection. Compounds 5–7, the 5'-deoxy, the tri-O-acetyl, and the 2'-deoxy derivatives of 1, respectively, were synthesized by glycosylation of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7-dione, the aglycon of 1, with the appropriate sugar moieties, utilizing the Vorbruggen procedure. Oxidative cleavage of the C_2–C_3 bond in 1 followed by reduction with sodium borohydride led to "seco" analogue 8. Nucleosides 2–8 were evaluated for antiviral activity in vivo against the Semliki Forest virus. The activity of compounds 2, 5, and 7 were similar to that of 1. Cyclic phosphate 3 was toxic at the high dose and weakly active at the lower dose. Compounds 4, 6, and 8 were inactive in this system.

Introduction

A recent report from our laboratories detailed the synthesis and immunopotentiating properties of purine nucleoside analogues in the thiazolo[4,5-*d*]pyrimidine ring system.[1] The guanosine analogue (1), in particular, was shown to possess excellent in vivo activity against a variety of DNA and RNA viruses.[1-4] While 1 exhibited a stimulatory effect on both cellular and humoral components of the immune response, the observed antiviral effect has been attributed primarily to the induction of α-interferon.[5] Other guanosine analogues and derivatives have been studied over the years for their ability to activate the immune system,[6] such as 8-bromo-, 8-mercapto-, and 7-methyl-8-oxoguanosine. These guanosines, including 1, have all been modified in the heterocyclic moiety relative to guanosine itself. It is evident from our own studies[1,7,8] that there is a good deal of "structural permissiveness" with regard to the structure–activity relationship and requirements for good in vivo antiviral activity. When the exception of one report dealing with B-cell activation,[9] to the best of our knowledge, no other reports have appeared in the literature which address the contribution and struc-



1 : $R_1 = R_2 = R_3 = OH$

2 : $R_1 = R_2 = OH$, $R_3 = -O-P(=O)(OH)OH$

3 : $R_1 = OH$, R_2, $R_3 = -O-P(=O)(OH)-O-$

4 : $R_1 = R_2 = OH$, $R_3 = -OSO_2NH_2$

5 : $R_1 = R_2 = R_3 = -OCOCH_3$

6 : $R_1 = R_2 = OH$, $R_3 = H$

7 : $R_1 = H$, $R_2 = R_3 = OH$

† Present address: Stratagene, La Jolla, CA 92037.
‡ Present address: Isis Pharmaceuticals, Carlsbad, CA 92008.
§ Present address: Utah State University, Logan, UT 84322.
∥ Present address: Triplex Pharmaceuticals, Woodlands, TX 77380.
⊥ Present address: Department of Medicine-0945, School of Medicine, University of California—San Diego, La Jolla, CA 92093-0945.

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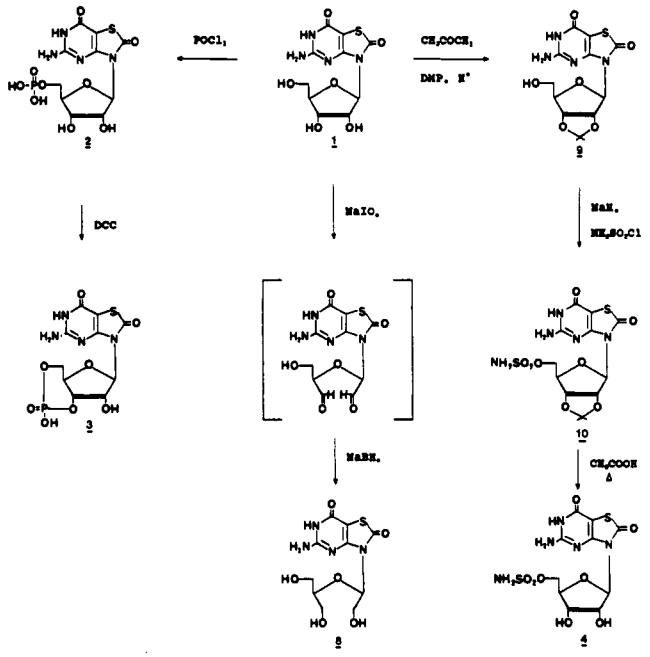
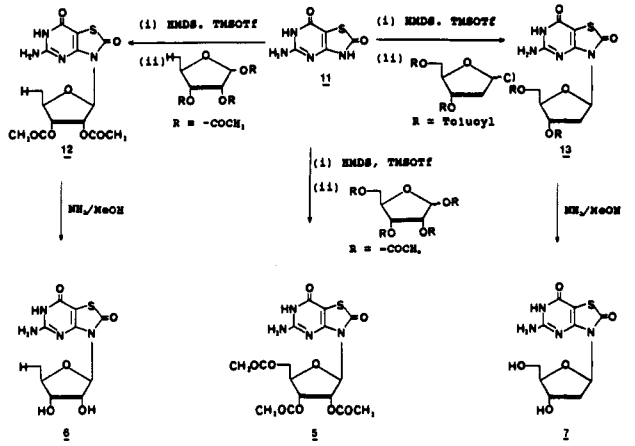
Scheme I



Scheme II



tural requirements for biological activity of the sugar moiety of these guanosines. To investigate this important aspect, the synthesis and biological evaluation of several guanosine analogues with modified sugars were undertaken. Accordingly, 5′-monophosphate **2** was synthesized as a marker for biochemical studies. The less ionic 3′,5′-cyclic monophosphate **3** and the nonionic 5′-*O*-sulfamoyl derivative (**4**) were prepared as "preactivated forms" of **1** which would more readily penetrate the cell membrane than monophosphate **2** itself. Tri-*O*-acetylated derivative **5** was synthesized as a potential prodrug form of **1**. 5′-Deoxyribosyl compound **6** was prepared to preclude the enzymatic activation via phosphorylation by a nucleoside kinase or by a reverse 5′-nucleotidase. 2′-Deoxyribosyl derivative **7** was then prepared to directly compare its activity to that of the corresponding ribosyl compound **1**. Finally, the "seco" or sugar ring-opened derivative **8** was synthesized as an acyclic analogue of **1**.

Chemistry

Scheme I outlines the synthesis of derivatives **2–4** and **8** where the nucleoside 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7-dione (**1**) served as the starting material. Phosphorylation of **1** with phosphorus oxychloride according to the model procedure of Yoshikawa et al.,[10] followed by purification with DEAE-cellulose chromatography, resulted in 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7-dione 5′-monophosphate ammonium salt (**2**) in 48% yield. Monophosphate **2** was subsequently cyclized with carbodiimide by using the method of Smith et al.[11] to the corresponding cyclic phosphate **3**, 5-amino-3-β-D-ribofuranosylthiazolo-[4,5-*d*]pyrimidine-2,7-dione 3′,5′-cyclic monophosphate, isolated as the ammonium salt. Nucleoside **1** was converted to the corresponding 2′,3′-O-isopropylidene derivative (**9**) by treatment with acetone and dimethoxypropane under acid-catalyzed conditions in 80% yield. Treatment of **9** with sulfamoyl chloride in the presence of sodium hydride led to sulfamoyl derivative **10**, which was subsequently deblocked by heating with aqueous acetic acid to yield 5′-*O*-sulfamoyl-5-amino-3-β-D-ribofuranosylthiazolo-[4,5-*d*]pyrimidine-2,7-dione (**4**). The structure of **4** was confirmed by X-ray crystallography, details of which will be published elsewhere.[12] Treatment of **1** with sodium periodate according to the procedure of Rossi and Lerner[13] followed by borohydride reduction of the dialdehyde intermediate led to "seco" analogue 2-*O*-[2-hydroxy-1(*R*)-(5-amino-2,3,6,7-tetrahydro-2,7-dioxothiazolo[4,5-*d*]pyri-

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Table I. Effects of Compounds 1-3 on a Semliki Forest Virus Infection in Mice

compd	dose,[a] mg/kg	survivors/total (%)	mean survival time,[b] days
placebo[c]		1/12 (8)	6.5 ± 1.1[d]
1	100	6/12 (50)	6.5 ± 0.5
1	50	3/12 (25)	8.8 ± 2.8[e]
1	25	3/12 (25)	7.2 ± 1.9
2	100	8/12 (67)[f]	6.5 ± 0.6
2	50	6/12 (50)	8.1 ± 1.5[e]
2	25	3/12 (25)	7.1 ± 2.1
3	100	1/2 (50)[g]	7.0 ± 0.0
3	50	2/12 (17)	7.1 ± 2.1
3	25	5/12 (42)	7.3 ± 1.3

[a] Half-daily doses were administered 24 and 18 h prior to virus inoculation. [b] Of mice that died. Survivors lived through 21 days. [c] A 2% sodium bicarbonate solution served as the placebo and as diluent for the compounds. [d] Standard deviation. [e] Statistically significant ($p < 0.05$)—determined by the two-tailed t-test. [f] Statistically significant ($p < 0.02$)—determined by the two-tailed Fisher exact test. [g] Ten mice died before virus inoculation of drug toxicity.

midin-2-yl)ethyl]glycerol (8).

Nucleosides 5–7 were synthesized starting with 5-aminothiazolo[4,5-*d*]pyrimidine-2,7-dione[14] (11), the aglycon of 1, as shown in Scheme II. Silylation of 11 followed by glycosylation with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose under Vorbruggen conditions[15] furnished 5-amino-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazolo-[4,5-*d*]pyrimidine-2,7-dione (5). Attempts to directly acylate 1 with acetic anhydride led to complex mixtures and very low isolated yields of 5. Compound 5 was synthesized as a possible prodrug of 1 and in vivo antiviral data (Table III) suggest that compound 5 was metabolized to 1.

In a manner similar to that of 5, glycosylation of 11 with 5-deoxy-1,2,3-tri-*O*-acetyl-D-ribofuranose[16] followed by ammonolysis of the 2′,3′-acetyl groups led to 5-amino-3-(5-deoxy-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7-dione (6). The 2′-deoxy derivative of 1 was synthesized by a fusion procedure in which compound 11 was silylated with hexamethyldisilazane and the silylated heterocycle fused with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[17] to yield the protected 2′-deoxynucleoside 13 as a mixture of α and β isomers. This mixture was deblocked with methanolic ammonia and a single product isolated by fractional crystallization. This product was identified by proton NMR[18] to be 5-amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7-dione (7), isolated in 42% yield.

Antiviral Activity

Compounds 2–8 were evaluated for activity against the Semliki Forest virus in an animal virus infection model.

Table II. Activities of Compounds against a Semliki Forest Virus Infection in Mice

compd	dose,[a] mg/kg	survivors/total (%)	mean survival time[b] days
placebo[c]		3/12 (25)	8.3 ± 2.2[d]
1	100	11/12 (92)[e]	10.0 ± 0.0
1	200	12/12 (100)[e]	>21
4	100	2/12 (17)	8.0 ± 2.3
4	200	1/12 (8)	8.2 ± 2.4
6	100	1/12 (8)	8.0 ± 2.3
6	200	0/12 (0)	8.4 ± 1.8
7	100	6/12 (50)	6.8 ± 0.8
7	200	10/12 (83)[e]	7.0 ± 0.0
8	100	1/12 (8)	7.0 ± 1.3
8	200	0/12 (0)	7.8 ± 1.9

[a] Half-daily doses were administered 24 and 18 h prior to virus inoculation. [b] Of mice that died. Survivors lived through 21 days. [c] A 2% sodium bicarbonate solution served as the placebo and as diluent for the compounds. [d] Standard deviation. [e] Statistically significant ($p < 0.02$)—determined by the two-tailed Fisher exact test.

Table III. Effects of 1 and 5 Given by Different Treatment Routes To Combat a Semliki Forest Virus Infection in Mice

compd (150 mg/kg)	treatment[a] route	survivors/ total (%)	mean survival time,[b] days
placebo[c]	ip	0/12 (0)	8.0 ± 2.23[d]
1	ip	10/11 (91)[e]	7.0 ± 0.0
1	sc	7/7 (100)[e]	>21
1	iv	8/11 (73)[e]	8.0 ± 1.7
1	po	3/12 (25)	7.1 ± 1.2
5	ip	11/12 (92)[e]	8.0 ± 0.0
5	sc	9/11 (82)[e]	7.0 ± 0.0
5	iv	5/12 (42)[e]	7.9 ± 2.4
5	po	2/12 (17)	6.6 ± 1.8

[a] Single treatments given 24 h prior to virus inoculation. ip = intraperitoneal; sc = subcutaneous; iv = intravenous; po = oral. [b] Of mice that died. Survivors lived through 21 days. [c] A 2% sodium bicarbonate solution served as the placebo and as diluent for the two compounds. [d] Standard deviation. [e] Statistically significant ($p < 0.05$)—determined by the two-tailed Fisher exact test.

The test compounds were evaluated in comparison with nucleoside 1. Table I shows the activity of 5′-monophosphate 2 and 3′,5′-cyclic phosphate 3, while the activities of 5′-sulfamoyl derivative 4, 5′-deoxy analogue 6, 2′-deoxy analogue 7, and "seco" analogue 8 are listed in Table II. Within limits of biological variability, compounds 2 and 7 showed similar antiviral activities compared to that of 1. The activity observed for 2 may likely be the result of the ability of this derivative to serve as a prodrug form of 1 via nucleotidase dephosphorylation at the cell membrane, thus allowing it to enter the cell as the free nucleoside 1. Cyclic phosphate 3, on the other hand, was overtly toxic at 100 mg/kg but showed weak antiviral effect at the lowest dose. The observed toxicity of 3, the reasons for which remain unclear, precluded further antiviral evaluation of this derivative. 5′-Deoxyribosyl derivative 6 was inactive, suggesting that activation via phosphorylation may be a requirement for activity in this system or that the 5′-OH group may play some other essential role. On the other hand, 5′-sulfamate 4 was likewise devoid of significant antiviral activity, suggesting that an intracellular phosphorylated form of 1 may not be the active form of the drug. Earlier studies from our laboratory have shown that aglycon 11 is not a substrate for hypoxanthine-guanine phosphoribosyltransferase and no intracellular phosphorylated forms of 1 were detected with ^{14}C-labeled 1.[19] "Seco" derivative 8 was also found to be

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inactive, thus suggesting that a ribosyl moiety is important for activity in this system.

Preliminary tests for the activity of the prodrug 5-amino-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)thiazolo-[4,5-d]pyrimidine-2,7-dione (5) indicated that its activity was comparable to that of 1. Hence it was further tested for antiviral activity via several routes of administration, the results of which are depicted in Table III. The activities of 1 and 5 were comparable when administered by four different treatment routes. In particular, prodrug 5 did not show any increased oral antiviral activity relative to that of 1. It should be noted here that all compounds studied were devoid of significant in vitro antiviral activity against Semliki Forest virus, suggesting that protection was conferred by immunopotentiation rather than by direct antiviral properties. Likewise, if the compounds were administered more than 24 h after virus inoculation, no protection was observed in the animals.

The Semliki Forest virus represents a class of alphaviruses transmitted by insect vectors. It is also one of the viruses against which the broad-spectrum antiviral agent ribavirin[20,21] is inactive. Alphaviruses are of medical importance in areas where insect-borne diseases prevail. Since induction of interferon is believed to be responsible for the antiviral activity of 1 and most likely the other active compounds presented here, these new analogues could exhibit activity against other interferon-sensitive viruses as well.

Experimental Section

Chemistry. Melting points were recorded on a Haake-Buchler digital melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on an IBM NR300AF spectrometer at 300.1 MHz. The chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane as internal standard. Ultraviolet spectra were recorded on a Beckman DU-50 spectrophotometer. Combustion analyses, reported for certain select intermediates, were performed by Robertson Laboratories, Florham Park, NJ. 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 chromatography.

Virus Infection Models. Swiss Webster female mice (Charles River Labs, Wilmington, MA), weighing about 20 g each at the beginning of the experiment, were administered compounds or placebo. The compounds were dissolved in 2% aqueous solution of sodium bicarbonate and injected intraperitoneally 24 and 18 h before virus inoculation, a regimen optimal for treatment against various RNA virsuses.[22] Viruses were pretitrated in the animals to identify doses which were 10 times the LD_{50}, and each experiment was conducted with 10 LD_{50}. Experiments ran for 21 days, at which time the animals were considered protected from the lethal phase of the infections. Statistical evaluations compared drug-treated groups to respective placebo controls. Increases in survival numbers were evaluated by the two-tailed Fisher exact test. Mean survival time increases were statistically analyzed by the two-tailed Student's *t* test.

5-Amino-3-(2,3-O-isopropylidene-β-D-ribofuranosyl)thiazolo[4,5-d]pyrimidine-2,7-dione (9). To an ice-cold suspension of 5-amino-3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine-2,7-dione[1] (1; 1.86 g, 6 mmol) in a mixture of acetone (100 mL) and dimethoxypropane (150 mL) was added perchloric acid (1.0 mL) dropwise, and the resulting mixture stirred at 0 °C for 30 min. The reaction mixture was neutralized while cold to pH 7 with 1 N sodium hydroxide. The resulting solution was concentrated in vacuo and the residue flash chromatographed on silica gel with 10% acetone in chloroform as eluent to yield the title compound as an amorphous solid (1.7 g, 80%): ^{1}H NMR (DMSO-d_6) δ 1.28, 1.47 (2 s, 6 H, isopropylidene methyls), 6.0 (d, 1 H, C_1'H), 7.0 (br s, 2 H, NH_2), 11.29 (s, 1 H, NH), and other sugar protons. Anal. $C_{13}H_{16}H_4SO_6$ (C, H, N, S).

5'-O-Sulfamoyl-5-amino-3-(2,3-O-isopropylidene-β-D-ribofuranosyl)thiazolo[4,5-d]pyrimidine-2,7-dione (10). To dry THF (100 mL) was added hexane-washed sodium hydride (0.61 g, 60% in oil). Nucleoside 9 (1.34 g, 3.8 mmol) was added and the resulting suspension cooled to 0 °C, with stirring. A solution of chlorosulfonamide (0.84 g, 7.3 mmol) in dry THF (20 mL) was added dropwise with stirring and the resulting mixture stirred at 0 °C for 4 h. The reaction mixture was quenched with methanol (10 mL), followed by the addition of a saturated aqueous solution of ammonium chloride (10 mL). The mixture was filtered, and the filtrate was adsorbed on silica gel and flash chromatographed with 20% acetone in chloroform as eluent to yield 1.31 g (80%) of the title compound as a foam: ^{1}H NMR (DMSO-d_6) δ 1.32, 1.51 (2 s, 6 H, isopropylidene methyls), 6.0 (s, 1 H, C_1H), 7.05 (br s, 2 H, NH_2), 7.58 (s, 2 H, SO_2NH_2), and other sugar protons. Anal. $C_{13}H_{17}N_5S_2O_8$ (C, H, N, S).

5-Amino-3-(2,3-di-O-acetyl-5-deoxy-β-D-ribofuranosyl)-thiazolo[4,5-d]pyrimidine-2,7-dione (12). A mixture of finely powdered and dry 5-aminothiazolo[4,5-d]pyrimidine-2,7-dione[14] (11; 2.5 g, 13.6 mmol), hexamethyldisilazane (150 mL), and dry pyridine (5 mL) was heated to reflux (bath temperature 135 °C) for 8 h with exclusion of moisture. The mixture was concentrated in vacuo and the residual syrup was dissolved in anhydrous acetonitrile (75 mL). To the resulting clear solution was added 5-deoxy-1,2,3-tri-O-acetyl-D-ribofuranose[16] (4.5 g, 17.3 mmol) and the solution stirred for 10 min. Trimethylsilyl trifluoromethanesulfonate (4.5 mL, 20 mmol) was added and the mixture stirred at room temperature overnight. The mixture was concentrated in vacuo and the residue flash chromatographed over silica gel with an 8:1 mixture of chloroform–methanol as eluent to yield the title compound which crystallized from a mixture of acetone and hexane (1.25 g, 25%): mp 223 °C; UV (pH 1) 214, 244, 299 nm; (pH 7) 209, 245, 289 nm; (pH 11) 213, 245, 297 nm; ^{1}H NMR ($CDCl_3$) δ 1.42 (d, 3 H, C_5'-methyl), 2.13, 2.16, (2 s, 6 H, acetyl), 4.19 (m, 1 H, C_4'H), 5.36, 5.85 (2 m, 2 H, C_2'H and C_3'H), 6.00 (d, 1 H, J = 3.1 Hz, C_1'H), 6.05 (br s, 2 H, NH_2), 11.5 (br s, 1 H, NH). Anal. $C_{14}H_{16}N_4O_7S$ (C, H, N, S).

5-Amino-3-(2-deoxy-3,5-di-O-p-toluoyl-β-D-*erythro*-pentofuranosyl)thiazolo[4,5-d]pyrimidine-2,7-dione (13). A mixture of 5-aminothiazolo[4,5-d]pyrimidine-2,7-dione[14] (11; 4.3 g, 23.3 mmol) and trimethylsilyl trifluoromethanesulfonate (13.6 mL, 73.4 mmol) in hexamethyldisilazane (70 mL) was refluxed for 3 h. The mixture was concentrated in vacuo and the residue fused with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-D-*erythro*-pentofuranose[17] (11.8 g, 73 mmol) at 110 °C for 30 min. The resulting product mixture was dissolved in ethyl acetate (400 mL) and poured into a stirring 5% aqueous solution of sodium bicarbonate (250 mL). The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was flash chromatographed over silica gel with 20% acetone in dichloromethane as eluent to yield 13 (1.7 g, 14%) as a mixture of α and β isomers: mp 268–270 °C; UV (pH 1) 243, 300 nm; (pH 7) 243, 302 nm; (pH 11) 243, 284 nm; ^{1}H NMR (DMSO-d_6) δ 6.37 (t, 1 H, C_1'H), 7.0 (br s, 2 H, NH_2), 7.2, 7.9 (2 m, 5 H, aromatics), 11.35 (br s, 1 H, NH), and other sugar protons.

5-Amino-3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine-2,7-dione 5'-Monophosphate Ammonium Salt (2). A suspension of 5-amino-3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine-2,7-dione[1] (1; 2.1 g, 6.6 mmol) in freshly distilled trimethyl phosphate (25 mL) was cooled to −20 °C. Two equal portions of phosphorus oxychloride (0.64 mL, 6.6 mmol) was added within an interval of 1 h. The mixture was stirred at −5 °C for 2 h and then poured into anhydrous diethyl ether (150 mL). The mixture was centrifuged at 6000 rpm (− 5 °C) for 10 min and the ether

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layer decanted off. Ice–water (100 mL) was added to the residual oil and the resulting solution neutralized to pH 7.5 by the addition of aqueous ammonium bicarbonate. The solution was then applied to a DEAE-cellulose column (3.2 × 35 cm), washed with water, and eluted with a gradient (0.0–0.25 M, 2 L) of aqueous ammonium bicarbonate. During the water wash, unreacted starting material (0.5 g) was recovered. The appropriate fractions were pooled and concentrated in vacuo. The residue was dissolved in water and the solution lyophilized to yield **2** (1.01 g, 48% based on reacted starting material): mp 190–194 °C; UV (pH 1 and 7) 243, 301 nm; (pH 11) 243, 289 nm; ^{1}H NMR (DMSO-d_6) δ 5.71 (s, 1 H, $C_{1'}$H), 7.15 (br s, 5 H, NH_2 and NH_4^+), 11.25 (br s, 1 H, NH), and other sugar protons. Anal. $C_{10}H_{13}N_4O_9SP\cdot NH_3$ (C, H, N, S).

5-Amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7-dione 3′,5′-Cyclic Monophosphate Ammonium Salt (3). To a solution of **2** (1.02 g, 2.28 mmol) in water (10 mL) and pyridine (3 mL) was added morpholinodicyclohexylcarbodiimide (0.67 g, 2.28 mmol). The mixture was concentrated in vacuo and coevaporated with pyridine (3 × 10 mL). The resulting syrup was dried over phosphorus pentoxide under vacuum overnight. The dried syrup was dissolved in dry pyridine (100 mL) and added dropwise to a refluxing solution of dicyclohexylcarbodiimide (25 g) in pyridine (300 mL). After the addition was complete, the resulting solution was refluxed for 2 h and stirred at room temperature overnight. The solution was concentrated to dryness in vacuo and the residue was partitioned between water (150 mL) and ether (150 mL). The aqueous layer was separated and its pH adjusted to 7.7 with aqueous ammonium bicarbonate. The solution was then concentrated in vacuo to about 100 mL and applied to a DEAE-cellulose column (3.2 × 30 cm). The column was eluted initially with water followed by a gradient of aqueous ammonium bicarbonate (0.0–0.2 M; 2 L). The appropriate fractions were pooled, concentrated in vacuo, and lyophilized several times to yield 0.2 g (22%) of the title compound: mp >244 °C (dec); UV (pH 1 and 7) 243, 300 nm; (pH 11) 243, 289 nm; ^{1}H NMR (DMSO-d_6) δ 5.60 (d, 1 H, $C_{2'}$-OH), 5.72 (s, 1 H, $C_{1'}$H), 7.15 (br s, 6 H, NH_2 and NH_4^+), 11.40 (br s, 1 H, NH), and other sugar protons. Anal. $C_{10}H_{11}N_4O_8SP\cdot NH_3\cdot 1.25H_2O$ (C, H, N, S, P).

5′-*O*-Sulfamoyl-5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7-dione (4). A solution of 5′-*O*-sulfamoyl-5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7-dione (**10**; 1.3 g, 3.0 mmol) in 80% acetic acid (50 mL) was heated at 100 °C with stirring for 3 h. The solution was concentrated in vacuo and the residue coevaporated with methanol (50 mL). The resulting solid was crystallized from aqueous ethanol to give analytically pure **4** (0.71 g, 60%) as a crystalline solid; mp 203–205 °C dec; UV (pH 1) 216 nm; (pH 7) 215 nm; (pH 11) 290 nm; ^{1}H NMR (DMSO-d_6) δ 4.0 (m, 2 H), 4.2 (m, 2 H), 4.7 (m, 1 H), 5.2, 5.4 (2 d, 2 H, 2-OH), 5.82 (d, 1 H, J = 4.2 Hz, $C_{1'}$H), 7.0 (br s, 2 H, NH_2), 7.55 (br s, 2 H, SO_2NH_2). Anal. $C_{10}H_{13}N_5S_2O_8$ (C, H, N, S).

5-Amino-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7-dione (5). A mixture of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7-dione[14] (**11**; 10 g, 54.4 mmol), hexamethyldisilazane (100 mL), ammonium sulfate (0.015 g), and pyridine (10 mL) was heated at reflux for 4 h with exclusion of moisture. The mixture was concentrated to dryness in vacuo and the residue was dissolved in dry acetonitrile (300 mL). To the resulting solution was added 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (20.7 g, 65.1 mmol) followed by trimethylsilyl trifluoromethanesulfonate (17 mL, 76.6 mmol) and the mixture stirred at room temperature for 16 h. The mixture was concentrated to dryness in vacuo and the residue was dissolved in ethyl acetate (750 mL). The organic solution was washed with 5% aqueous sodium bicarbonate (2 × 200 mL), dried over anhydrous sodium sulfate, and evaporated to dryness in vacuo. The residual syrup was flash chromatographed over silica gel with 10% methanol in dichloromethane as eluent to yield 7.1 g (29%) of **5** as a foam; ^{1}H NMR (DMSO-d_6) δ 2.01–2.08 (3 s, 9 H, acetyls), 5.95 (d, 1 H, $C_{1'}$H), 7.04 (br s, 2 H, NH_2), 11.35 (br s, 1 H, NH), and other sugar protons. Anal. $C_{16}H_{18}N_4O_9S$ (C, H, N, S).

5-Amino-3-(5-deoxy-β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7-dione (6). A solution of **12** (0.45 g, 1.17 mmol) in methanol saturated with ammonia at 0 °C was stirred in a pressure bottle at room temperature overnight. The mixture was concentrated in vacuo and the residue was crystallized from ethyl acetate to yield **6** (0.30 g, 85%) as a crystalline solid; mp 197 °C; UV (pH 1) 215, 245, 299 nm; (pH 7) 215, 243, 299 nm; (pH 11) 209, 244, 289 nm; ^{1}H NMR (DMSO-d_6) δ 1.19, 1.21 (d, 3 H, $C_{5'}$-methyl), 3.75 (m, 1 H, $C_{4'}$H), 3.93, 4.66 (2 m, 2 H, $C_{2'}$H and $C_{3'}$H), 4.91, 5.27 (2 d, 2 H, OH), 5.73 (d, 1 H, J = 4.0 Hz, $C_{1'}$H), 6.98 (br s, 2 H, NH_2), 11.28 (bs, 1 H, NH). Anal. $C_{10}H_{12}N_4O_5S\cdot {}^1/_4CH_3OH$ (C, H, N).

5-Amino-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7-dione (7). Sodium methoxide (0.12 g, 2.2 mmol) was added to a solution of 5-amino-3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7-dione (**13**; 0.47 g, 0.87 mmol) in anhydrous methanol (100 mL) and the resulting solution stirred at room temperature for 8 h. The solution was neutralized with Dowex 50W-X8 (H^+ form) resin and filtered and the filtrate concentrated in vacuo. The residue was triturated with anhydrous ether (35 mL) whereupon a solid formed. The solid was crystallized from ethanol to yield **7** (0.11 g, 42%): mp >170 °C (sinters); ^{1}H NMR (DMSO-d_6) δ 4.68 (t, 1 H, $C_{3'}$-OH), 5.20 (d, 1 H, $C_{5'}$-OH), 6.24 (t, 1 H, J = 7.2 Hz, $C_{1'}$H), 6.94 (br s, 2 H, NH_2), 11.23 (br s, 1 H, NH), and other sugar protons. Anal. $C_{10}H_{12}N_4O_6S$ (C, H, N, S).

2-*O*-[2-Hydroxy-1(*R*)-(5-amino-2,3,6,7-tetrahydro-2,7-dioxothiazolo[4,5-*d*]pyrimidin-3-yl)ethyl]glycerol (8). To a suspension of **1** (1.0 g, 3.2 mmol) in water (25 mL), cooled in an ice bath, was added sodium periodate (0.68 g, 3.2 mmol) in small portions, and the resulting mixture was stirred at room temperature overnight. The mixture was poured into absolute ethanol (50 mL), stirred for 0.5 h, and filtered. The filtrate was concentrated in vacuo, the residue dissolved in water (25 mL), and this solution added dropwise to a solution of sodium borohydride (0.64 g) in water (15 mL). The resulting solution was stirred for 2 h at room temperature, acidified with Dowex 50W-X8 (H^+ form) resin, and filtered and the filtrate concentrated in vacuo. The residue was coevaporated several times with absolute methanol. The resulting residue was crystallized from hot ethanol to yield 0.34 g (33.4%) of the title compound: mp 220 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.35 (m, 6 H, $C_{2'}$, $C_{3'}$ and $C_{5'}$ methylenes), 3.85 (m, 1 H, $C_{4'}$H), 4.37, 4.62 (2 t, 2 H, $C_{3'}$-OH and $C_{5'}$-OH), 5.09 (t, 1 H, $C_{2'}$-OH), 5.72 (t, 1 H, $C_{1'}$H), 6.90 (s, 2 H, NH_2), 11.17 (s, 1 H, NH). Anal. $C_{10}H_{14}N_4O_6S$ (C, H, N, S).

Registry No. **1**, 122970-40-5; **2**, 135505-24-7; **3**, 135505-25-8; **4**, 135505-26-9; **5**, 124737-24-2; **6**, 135505-27-0; **7**, 124737-22-0; **8**, 135505-28-1; **9**, 124737-25-3; **10**, 135505-29-2; **11**, 30161-97-8; **12**, 135505-30-5; β-**13**, 124737-27-5; α-**13**, 135505-31-6; 1-chloro-2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranose, 3601-89-6; 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose, 28708-32-9; 5-deoxy-1,2,3-tri-*O*-acetyl-D-ribofuranose, 37076-71-4.