Synthesis and Single-crystal X-ray Diffraction Analysis of 2-Sulfamoyladenosine (6-Amino-9-β-D-ribofuranosylpurine-2-sulfonamide)

Naeem B. Hanna, Steven B. Larson, Roland K. Robins and Ganapathi R. Revankar*†

ICN Nucleic Acid Research Institute, 3300 Hyland Avenue,
Costa Mesa, California 92626

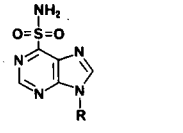
Received January 18, 1990

2-Amino-9-β-D-ribofuranosylpurine-2-sulfonamide (2-sulfamoyladenosine, **4**), a congener of sulfonosine (**3**), was synthesized by four different routes. Acid catalyzed fusion of 6-chloropurine-2-sulfonyl fluoride (**5**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**) gave a good yield of 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonyl fluoride (**9**). Ammonolysis of **9** furnished **4**. Lewis acid catalyzed glycosylation of the trimethylsilyl derivative of either 6-chloropurine-2-sulfonamide (**6**) or 6-aminopurine-2-sulfonamide (**7**) with **8** gave the corresponding *N*9-glycosylated products, **10** and **11**, respectively, which on ammonolysis gave **4**. Amination of 2-thioadenosine (**12**) with chloramine solution gave the sulfenamide derivative **13**, which on subsequent oxidation with *m*-chloroperoxybenzoic acid furnished an alternate route to **4**. The structure of **4** was established by single-crystal X-ray diffraction studies. 2-Sulfamoyladenosine (**4**) is devoid of significant inhibitory activity against L1210 leukemia in mice.

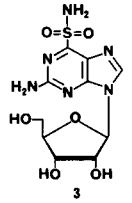
J. Heterocyclic Chem., **27**, 1713 (1990).

6-Mercaptopurine (6MP) is one of the oldest anticancer agents currently in clinical use. Introduced more than 35 years ago [1], 6MP and its 2-amino derivative, 6-thioguanine (6TG) were initially shown to be efficacious in the treatment of acute leukemia in children and adults [2,3]. Today, these thiopurine bases remain valuable agents for the induction and maintenance of remissions in patients with acute myelocytic (AML) and acute lymphocytic leukemia (ALL).

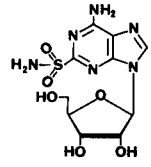
Despite their proven clinical importance, 6MP and 6TG have certain therapeutic disadvantages [4-7] which have continued to stimulate the search for purine derivatives with enhanced therapeutic efficacy. In this regard, purine-6-sulfonamide (**1**) first synthesized and reported from our laboratory [8], exhibited significant antitumor activity against Adenocarcinoma 755 and L1210 leukemia in mice at several dosage levels [9-11]. Compound **1** at 75 mg/kg per day x 5 against L1210 leukemia showed a T/C of 165. The ribonucleoside of purine-6-sulfonamide (**2**), also prepared and reported recently from our laboratory [12], exhibited a T/C of 147 against L1210 leukemia in mice at 173 mg/kg dosage and reduced body burdens of viable L1210 leukemia cells by more than 98.3% [12]. However, administered qd (once daily) on day one, 2-amino-9-β-D-ribofuranosylpurine-6-sulfonamide (sulfonosine, **3**) gave a T/C of 128 at 62 mg/kg dosage level [12]. Thus, it is of particular interest that a subtle change to an oxidized sulfur atom in the form of sulfonamide at position 6 resulted in a new group of purine derivatives possessing significant antitumor properties. In an attempt to define the individual structural features that influence the antitumor profile of this new class of compounds, we have now synthesized 6-amino-9-β-D-ribofuranosylpurine-2-sulfonamide (2-sulfamoyladenosine, **4**) and evaluated it for antileukemic activity in mice.



1, R = H
2, R = β-D-ribofuranose



3
Sulfonosine



4
2-Sulfamoyladenosine

Table 1
Positional and Isotropic Thermal Parameters for all Atoms in **4**

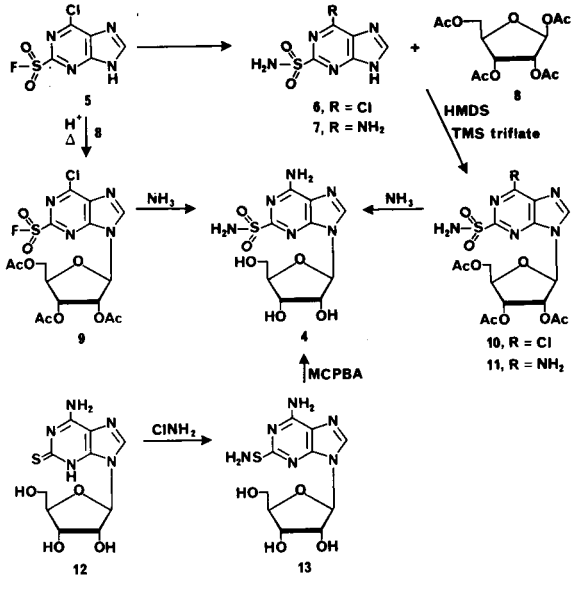
Atom	x/a	y/b	z/c	U_{eq} [a]
N1	.3867(5)	.3175(3)	.8448(3)	.0353(5)
C2	.4496(5)	.3303(3)	.6890(3)	.0326(5)
N3	.6188(5)	.4339(3)	.5674(3)	.0324(5)
C4	.7503(5)	.5339(3)	.6235(3)	.0304(5)
C5	.7185(5)	.5359(3)	.7812(3)	.0319(5)
C6	.5157(5)	.4245(3)	.8958(3)	.0329(5)
N7	.8936(5)	.6536(3)	.7923(3)	.0390(6)
C8	1.0267(5)	.7202(3)	.6426(3)	.0373(6)
N9	.9458(5)	.6549(3)	.5343(2)	.0322(5)
S10	.2968(4)	.1721(2)	.6342(2)	.0397(2)
O11	.0151(5)	.1393(3)	.7165(3)	.0575(7)
O12	.3494(8)	.2239(4)	.4613(3)	.0827(10)
N13	.4649(5)	.0046(3)	.7082(3)	.0462(7)
N14	.4483(6)	.4200(3)	1.0487(3)	.0424(6)
C1'	1.059101	.695601	.360209	.0307(5)
C2'	.8306(5)	.7018(3)	.2554(3)	.0325(5)
C3'	.9449(5)	.8378(3)	.0964(3)	.0356(6)
C4'	1.0741(5)	.9578(3)	.1586(3)	0.353(6)
C5'	.8682(7)	1.0837(4)	.1921(4)	.0583(9)
O2'	.7892(5)	.5521(3)	.2279(3)	.0462(5)
O3'	1.1585(5)	.7777(3)	.0029(3)	.0452(5)
O4'	1.1804(5)	.8552(3)	.3059(3)	.0402(5)
O5'	.9888(6)	1.1964(3)	.2460(4)	.0556(9)
O5'D	.696(3)	1.012(2)	.3403(13)	.069(5)
H8	1.161(6)	.802(4)	.613(4)	.048(7)
H13A	.395(6)	-.051(4)	.808(4)	.041(6)
H13B	.658(9)	.014(5)	.689(5)	0.77(11)

H14A	.310(8)	.363(5)	1.103(5)	.062(9)
H14B	.550(9)	.473(5)	1.087(5)	.072(11)
H1'	1.205(5)	.619(3)	.349(3)	.027(5)
H2'	.676(6)	.745(3)	.296(3)	.037(6)
H3'	.803(6)	.900(3)	.033(3)	.037(6)
H4'	1.247(6)	1.011(4)	.084(4)	.047(7)
HO2'	.743(7)	.489(4)	.324(4)	.044(6)
HO3'	1.060(10)	.716(6)	-.034(6)	.083(11)
H5'A	.707(2)	1.031(3)	.278(2)	.049(6)
H5'B	.791(2)	1.153(3)	.0908(14)	.049(6)
HO5'	.987(11)	1.137(7)	.335(7)	.082(11)
H5'AD	.740(5)	1.124(2)	.109(3)	.049(6)
H5'BD	.962(5)	1.1801(12)	.203(3)	.049(6)

[a] For non-hydrogen atoms, except O5'D which was refined isotropically, U is $U_{eq} = 1/3\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* A_{ij}$, where A_{ij} is the dot product of the i^{th} and j^{th} direct-space unit-cell vectors.

The subject ribonucleoside (2-sulfamoyladenosine) was synthesized by four different routes and the structure was established by single-crystal X-ray diffraction studies. The first method involved the acid catalyzed fusion of the viable precursor 6-chloropurine-2-sulfonyl fluoride (**5**) with a fully acylated sugar. Compound **5** was prepared as reported previously [8] and fused at 145° with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**) in the presence of bis(*p*-nitrophenyl) phosphate. An intractable reaction mixture was obtained from which the desired 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonyl fluoride (**9**) was isolated in 71% yield after silica gel column chromatography. No attempt was made to isolate and identify the other reaction products. Ammonolysis of **9** with liquid ammonia at 100° for 6 hours gave 2-sulfamoyladenosine (**4**) in 58% yield as needles.

Scheme I



The second procedure involved the Lewis acid catalyzed glycosylation of the trimethylsilyl derivative of 6-chloropurine-2-sulfonamide with a fully protected sugar. 6-Chloropurine-2-sulfonamide (**6**) [8] was silylated with hexamethyldisilazane (HMDS) in the presence of ammonium sulfate to give the silylated derivative. Reaction of this silylated derivative with one molar equivalent of **8** in anhydrous acetonitrile in the presence of 1.44 molar equivalent of trimethylsilyl trifluoromethanesulfonate (trimethylsilyl triflate) according to the general procedure of Vorbrüggen *et al.* [13] afforded 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonamide (**10**). The yield of **10** by this procedure was also 71%.

A similar glycosylation of the trimethylsilyl derivative of 6-aminopurine-2-sulfonamide (**7**) [8] with **8** furnished a 53% yield of 6-amino-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonamide (**11**) as white foam. Ammonolysis of either **10** or **11** with ethanolic ammonia at 0-4° at room temperature, readily gave the desired nucleoside **4** in good yield. It is worth mentioning that during ammonolysis, the C2-sulfamoyl group of **10** or **11** was not displaced by an amino group as it did with C6-sulfamoyl isomer [12]. C2-Sulfamoyl group in **10** or **11** seems to be quite resistant to ammonolysis.

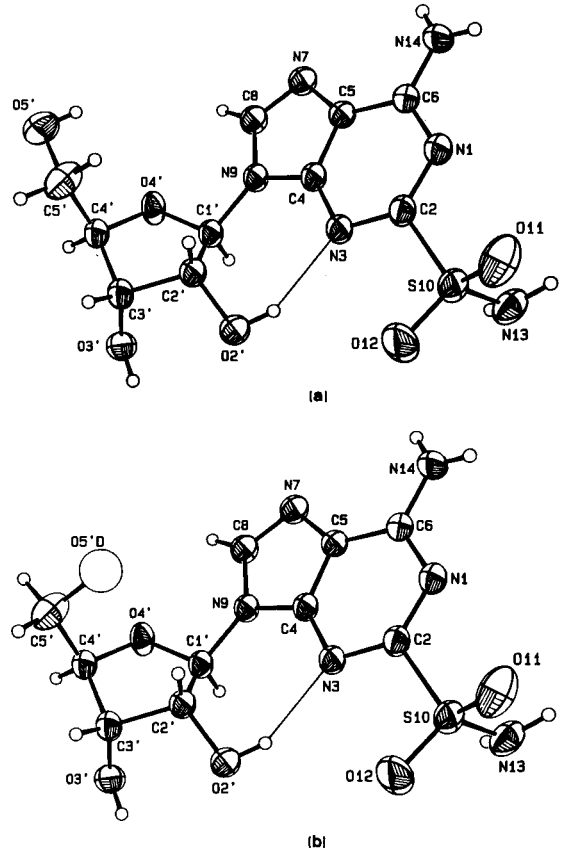


Figure 1. Prespective drawings of **4** illustrating atom labeling and the intramolecular hydrogen bond, 02'H02'...N3 (thin lines). The molecule is disordered at the 05' position with (a) 86% *trans-gauche* and (b) 14% *gauche-gauche*.

Table 2

Bond Lengths (Å), Bond Angles (°) and Selected Torsion Angles in **4**

1	2	3	1 – 2	1-2-3	1	2	3	1 - 2	1-2-3
C2	N1	C6	1328(4)	117.5(2)	N3	C2	S10	1.324(3)	113.5(2)
N3	C2	N1		131.2(3)	S10	C2	N1	1.804(4)	115.1(2)
C4	N3	C2	1.346(4)	109.6(2)	C5	C4	N9	1.382(4)	106.0(2)
C5	C4	N3		127.4(2)	N9	C4	N3	1.374(3)	126.5(2)
C6	C5	N7	1.419(3)	133.0(3)	C6	C5	C4		116.3(3)
N7	C5	C4	1.380(4)	110.6(2)	N14	C6	N1	1.325(4)	119.1(2)
N14	C6	C5		123.3(3)	N1	C6	C5	1.359(4)	117.7(2)
C8	N7	C5	1.320(3)	104.1(3)	N9	C8	N7	1.368(4)	113.4(2)
C1'	N9	C4	1.474(2)	126.4(2)	C1'	N9	C8		127.6(2)
C4	N9	C8		105.9(2)	O11	S10	O12	1.430(3)	119.6(2)
O11	S10	N13		105.4(2)	O11	S10	C2		109.3(2)
O12	S10	N13	1.421(3)	109.4(2)	O12	S10	C2		105.9(2)
N13	S10	C2	1.606(3)	106.7(2)	C2'	C1'	O4'	1.533(3)	106.96(13)
C2'	C1'	N9		112.56(13)	O4'	C1'	N9	1.408(2)	107.62(14)
C3'	C2'	O2'	1.530(3)	110.9(2)	C3'	C2'	C1'		101.5(2)
O2'	C2'	C1'	1.412(4)	114.6(2)	C4'	C3'	O3'	1.513(4)	108.6(2)
C4'	C3'	C2'		101.7(2)	O3'	C3'	C2'	1.410(4)	111.5(2)
C5'	C4'	C4'	1.508(4)	111.5(2)	C5'	C4'	C3'		112.8(3)
O4'	C4'	C3'	1.456(3)	105.4(2)	O5'	C5'	C4'	1.393(5)	112.8(3)
O5'D	C5'	C4'	1.394(11)	109.7(7)	C1'	O4'	C4'		109.8(2)

Selected Torsion Angles

χ_{CN}	C4-N9-C1'-O4'	161.8(2)	χ'_{CN}	C8-N9-C1'-O4'	-23.4(3)
ϕ_{oo}	O4'-C4'-C5'-O5'	62.9(3)	ϕ_{co}	C3'-C4'-C5'-O5'	-178.7(2)
ϕ_{oo}	O4'-C4'-C5'-O5'D	-40.0(8)	ϕ_{co}	C3'-C4'-C5'-O5'D	78.3(8)
Θ_0	C1'-C2'-C3'-C4'	-37.1(2)	Θ_1	C2'-C3'-C4'-04'	33.6(2)
Θ_2	C3'-C4-O4'-C1'	-16.4(2)	Θ_3	C2'-C1'-O4'-C4'	-8.0(2)
Θ_4	O4'-C1'-C2'-C3'	28.7(2)			

The title compound was also prepared by the sequential amination and controlled oxidation of 2-thioadenosine (**12**). Compound **12** was prepared as reported [14,15]. Thus, treatment of 2-thioadenosine (**12**) with an aqueous chloramine solution [12] [prepared from cold commercial sodium hypochlorite (clorox) and ammonium hydroxide solution] at ambient temperature, and purification of the reaction product by silica gel column chromatography provided a 51% yield of 6-amino-9-β-D-ribofuranosylpurine-2-sulfenamide (**13**). Oxidation of **13** with four molar equivalent of *m*-chloroperoxybenzoic acid (MCPBA) in ethanol gave the fully oxidized product 2-sulfamoyladenosine (**4**) in a 58% yield.

Single-crystal X-ray Diffraction Analysis of 2-Sulfamoyladenosine (**4**).

Atomic coordinates and isotropic thermal parameters are listed in Table 1. The bond lengths, bond angles and selected torsion angles are given in Table 2. The crystal structure is disordered at the 05′ position; the two molecular conformations are illustrated in Figure 1 which also details the atomic labeling. The structural determination confirms that compound **4** is an N9-glycosylated product with the β-anomeric configuration and, thus, it is a 2-substituted adenosine derivative. As such, a comparison to adenosine is relevant [16]. Bond lengths between the two structures are not significantly different. Thus, the root-mean-square deviation is 0.005 Å for sugar bond lengths (excluding the disorder-shortened C5′-05′ bond lengths) and 0.007 Å for the adenine system; the maximum differences of 0.012 Å are observed in the C2-N1 and N7-C8 bonds. Bond angles in the rigid purine rings are comparable except at C2. The 2-sulfamoyl group in **4** increases the N1-C2-N3 angle by 2.3° while a decrease is observed in the angles at the adjacent nitrogen atoms. The furanose-ring interior angles are comparable as well, but the angles involving the furanose-attached groups show differences up to 5.7°.

The glycosidic conformation is *anti* for both **4** and adenosine. The glycosidic torsion angle, X'_{CN}, of -23.4(3)° represents a slight 33.3° rotation of the purine ring about the C1′-N9 bond from the conformation of adenosine observed in the solid state. The sugar conformation, on the other hand, is very distinct from that found in adenosine. Thus, a C_3-*exo*/C_2-*endo* ($_3T^2$) conformation is

observed in **4** versus a C_3-*endo* conformation in adenosine. The pseudorotation angle (P) and amplitude of pucker (τ_m) [17] are 186.6° and 37.4° (versus 7.2° and 36.0° in adenosine). The major population (86%) of the disordered C5′-05′ side-chain has the *guache-trans* orientation found in adenosine; the other orientation (14%) is *gauche-gauche*.

The purine ring is slightly non-planar [rmsd: 0.023(2) Å]; the dihedral angle between the planar imidazole ring and the slightly non-planar pyrimidine ring is 1.92(10)°. The sulfonamido group is essentially equivalent in geometry to the sulfonamido group in the isomer 2-amino-9-β-D-ribofuranosylpurine-6-sulfonamide [12], although the S-C and S-N bonds are 0.014 Å longer in the title compound. Atom 012 lies in the purine plane [0.032(3) Å deviation] *cis* to N3; in the 6-sulfamoyl isomer, an oxygen is also approximately coplanar with the purine ring. The S-amino group in pyramidal [N13 is 0.250(3) Å out of the plane of the attached atoms] whereas the 6-amino group is planar since the nitrogen is conjugated with the purine ring.

The combination of C_3-*exo*/C_2-*endo* sugar pucker, which places both the adenine moiety and the 2′-hydroxyl group in equatorial positions and the *anti* glycosidic conformation results in an unusual intramolecular hydrogen bond (02′-H02′...N3; see Table 3) which forms a seven-membered (N3, C4, N9, C1′, C2′, 02′, H02′) ring. Atoms C2′ and 02′ are 0.894(2) and 0.265(2) Å above the mean plane of the other five atoms. The angles C4-N9-C1′, N9-C1′-C2′ and C1′-C2′-02′ are enlarged by 1.0-5.1° when compared to the non-hydrogen-bonded adenosine as if such compensation were necessary to accommodate the moderately strong intramolecular interaction.

Table 3 gives a complete list of the probable hydrogen bonding interactions. N1, N9 and 04′ are the only nitrogen and oxygen atoms that do not participate in hydrogen bonding. The most dubious entry, as determined by the H...A distance, is the 05′...012 interaction; however, the D...A distance suggests such an interaction and the H05′ position has a high uncertainty and represents a partial occupancy as well. The disordered 05′D appears to interact with 012 of a different molecule. Thus, rotation of the 5′-hydroxyl group about the C4′-C5′ bond places it within hydrogen-bonding distance of two distinct acceptors, which accounts for the observed disorder. Figure 2 is a view of the cell prependicular to the adenine plane. Besides illustrating the hydrogen-bonding network, the figure shows that the base rings of molecules translated along the α-axis are partially stacked in a staircase-like fashion such that N1 and C2 of one molecule lie over the cavity of the imidazole ring of the neighboring molecule. In addition, 011 of the sulfamoyl group lies 3.07 Å above the C2-N3 bond of an adjacent molecule. The interplanar stacking distance is about 3.57 Å, the same as observed in adenosine.

Antitumor Evaluation.

2-Sulfamoyladenosine (**4**) was evaluated [18] for the anti-L1210 activity in BDF_1 mice, along with the aglycon

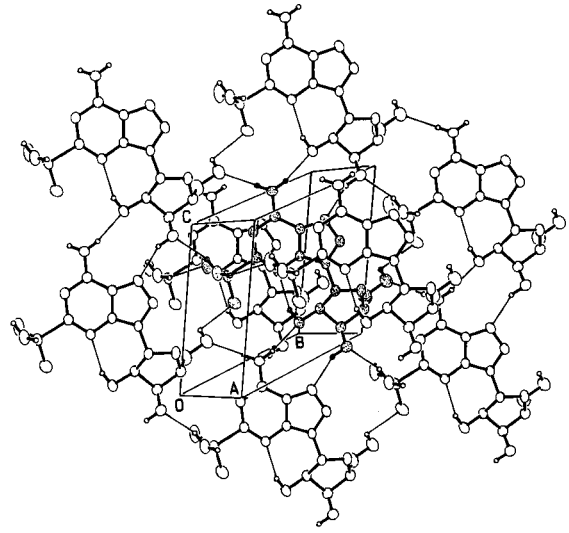


Figure 2. Perspective drawing of the unit cell of **4** viewed perpendicular to the adenine plane of the shaded molecule. All hydrogen bonding interactions involving the shaded molecule are indicated by thin lines. The partial base stacking of molecules translated along the *a*-axis is clearly visible.

Table 3
Hydrogen Bonding in **4**

D – H ••• A	Symmetry of A relative to D	d(D•••A) (Å)	d(H•••A) (Å)	∠(D-H••A) (°)
N13 H13A O3′	x-1.0,y-1.0,z+1.0	2.893(4)	2.06(3)	160.(3)
N13 H13B O11	x+1.0,y,z	2.961(4)	2.16(4)	145.(4)
N14 H14A O5′	x-1.0,y-1.0,z+1.0	2.922(4)	2.10(4)	174.(4)
N14 H14B O2′	x,y,z+1.0	2.943(4)	2.10(4)	167.(4)
O2′ HO2′ N3	x,y,z	2.817(4)	2.03(4)	155.(3)
O3′ HO3′ N7	x,y,z-1.0	2.899(4)	2.07(5)	156.(4)
O5′ HO5′ O12	x+1.0,y+1.0,z	2.838(4)	2.48(6)	153.(4)
O5′D ••• O12	x,y+1.0,z	2.785(15)	•••	•••

2-sulfamoyladenine (**7**) and the nucleoside precursor 6-amino-9-β-D-ribofuranosylpurine-2-sulfenamide (**13**). Administered qd (once daily) on day 1 at 800 mg/kg dosage (maximum solubility in water), none of the three compounds exhibited biologically significant antileukemic activity; *i.e.*, none produced a T/C $\geq$125, which is considered biologically significant. Percentage T/C were calculated according to the following equation: mean life span of treated mice/mean life span of control mice x 100. The observed lack of antileukemic activity of 2-sulfamoyl adenosine in mice indicates that the location of the sulfamoyl group in the purine ring (*e.g.*, at 6-position) is probably important for the antitumor activity.

Table 4
Crystal and Experimental Data [a,b] for **4**

Empirical formula	$C_{10}H_{14}N_6O_6S$
Formula weight	346.32
Crystal system	triclinic
Space group	P1
a (Å)	4.8254(5)
b (Å)	8.5704(8)
c (Å)	8.8214(11)
α (°)	70.265(15)
β (°)	81.112(10)
γ (°)	87.564(7)
V (Å^3)	339.24(7)
Z	1
ρ_{calcd} (g cm^{-3})	1.695
F(000) (electrons)	180
Radiation, λ (Å)	CuKα, 1.54178
Crystal dimensions (mm)	0.38x0.31x0.26x0.14
Crystal volume (mm^3)	0.0104
μ (cm^{-1})	25.216
Max 2θ (°)	152
Total refls, measd, unique	2814, 2754
Observed refls ($F \geq 4\sigma_F$)	2740
No. of Variables	275
S(goodness of fit)	1.87
R, wR [c]	0.0278, 0.0435
Extinction parameter	7.7(6) x 10^{-6}
Max, ave Δ/σ	0.007, 0.0007
Max, min in Δρ map (e/Å^3)	0.38, -0.47

[a] Unit-cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections in the range 51.2<2θ<59.4°. [b] Intensity measurements were made on an Enraf-Nonius CAD4 automatic diffractometer equipped with a graphite monochromator using an ω-2θ scan procedure (scan range in ω: 1.0+0.15 tan Θ in deggrees) and variable scan speeds (2.4-8.3°/min). Data reduction was accomplished with the SDP-Plus program package [c] and included Lorentz, polarization, decay (range: 1.000-1.005) and absorption corrections (transmission factor range: 0.492-0.749). [c] Function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = (\sigma_F^2 + 0.0004F^2)^{-1}$. $\sigma_F = F\sigma_I/2I$ and $\sigma_I = (N_{pk} + N_{bg1} + N_{bg2})^{1/2}$

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. The presence of water as indicated by elemental analysis was verified by ^{1}H nmr spectroscopy. Thin layer chromatography (tlc) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (E. Merck; 230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside derivatives by tlc was made with uv light or with 10% sulfuric acid in methanol spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30°. Infrared (ir) spectra were recorded in potassium bromide with a Perkin-Elmer 1420 spectrophotometer and ultraviolet spectra (uv) were recorded on a Beckman DU-50 spectrophotometer. Nuclear magnetic resonance (^{1}H nmr) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as the internal standard (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad).

6-Chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonyl Fluoride (**9**).

A finely ground mixture of 6-chloropurine-2-sulfonyl fluoride [8] (**5**, 2.36 g, 10 mmoles) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**, 3.18 g, 10 mmoles) was fused with stirring at 145° in the presence of bis(*p*-nitrophenyl) phosphate (0.1 g) under reduced pressure for 1 hour. Chromatography of the resulting reaction mixture on a silica gel column (2.5 x 25 cm) using dichloromethane:methanol (99:1, 98:2, v/v) as the eluent provided pure product, which on crystallization from diethyl ether gave 3.5 g (71%) of **9**, mp 145-147°; ir: ν max 1050, 1120 (S=O), 1220, 1370 (O=S=O), 1750 (C=O of acetyls) cm^{-1}; uv: λ max (*p*H 1) 266 nm (ε 9,900); (*p*H 7) 266 nm (ε 10,000); (*p*H 11) 266 nm (ε 8,900); ^{1}H nmr (DMSO-d_6): δ 1.99, 2.06, 2.12 (3 s, 9 H, 3COC*H*$_3$), 4.29-4.52 (2 m, 3 H, C_4.*H* and C_5.C*H*$_2$), 5.64 (t, 1 H, C_3.*H*), 5.88 (t, 1 H, C_2.*H*), 6.45 (d, 1 H, $J_{1',2'}$ = 4.5 Hz, C_1.*H*) and 9.25 (s, 1 H, C_8*H*); ^{19}F (CFCl$_3$): 53.46 (SO$_2$*F*).

Anal. Calcd. for $C_{16}H_{16}FClN_4O_9S$ (494.83): C, 38.83; H, 3.26; N, 11.32; Cl, 7.16; F, 3.84; S, 6.48. Found: C, 39.01; H, 3.17; N, 11.25; Cl, 7.28; F, 3.71; S, 6.53.

6-Chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonamide (**10**).

A mixture of 6-chloropurine-2-sulfonamide [8] (**6**, 1.4 g, 6 mmoles), hexamethyldisilazane (HMDS, 20 ml) and ammonium sulfate (0.1 g) was heated under reflux (135°, oil bath temperature) for 20 hours. The excess of HMDS was removed by distillation under reduced pressure at 50°. The residual syrup was co-evaporated with toluene (2 x 25 ml) and held at high vacuum for 2 hours at 50°. To a stirred solution of the above dry silylated sulfonamide in anhydrous acetonitrile (50 ml) was added successively 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**, 1.9 g, 6 mmoles) and trimethylsilyl triflate (1.8 ml, 8.6 mmoles) at room temperature. After stirring for 2 hours at ambient temperature, the reaction mixture was evaporated. The residue was dissolved in ethyl acetate (150 ml) and washed with cold 5% aqueous solution of sodium bicarbonate (2 x 75 ml), followed by water (2 x 100 ml). After drying over anhydrous sodium sulfate, the solvent was evaporated and the residue was purified on a flash silica gel column (1.5 x 25 cm). The column was eluted successively with 200 ml portions of dichloromethane:methanol (98:2, 96:4, v/v). The homogeneous fractions were pooled and evaporated to yield 2.1 g (71%) of **10** as white foam; ir: ν max 1050, 1160 (S=O), 1230,

1360 (O=S=O), 1740 (C=O of acetyls), 3100-3550 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 266 nm (ε 8,300); (*p*H 7) 265 nm (ε 8,200); (*p*H 11) 270 nm (ε 8,000); ^1H nmr (DMSO-d_6): δ 2.02, 2.06, 2.13 (3 s, 9 H, 3 $COCH_3$), 4.21-4.46 (2 m, 3 H, $C_{4'}H$ and $C_{5'}CH_2$), 5.62 (t, 1 H, $C_{3'}H$), 5.93 (t, 1H, $C_{2'}H$), 6.38 (d, 1 H, $J_{1',2'}$ = 5.5 Hz, $C_{1'}H$), 7.82 (s, 2 H, SO_2NH_2) and 9.11 (s, 1 H, C_8H).

Anal. Calcd. for $C_{16}H_{18}ClN_5O_9S$ (491.85): C, 39.07; H, 3.69; N, 14.24; Cl, 7.21; S, 6.52. Found: C, 38.91; H, 3.54; N, 13.99; Cl, 6.92; S, 6.22.

6-Amino-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2-sulfonamide (**11**).

In a similar manner as described for **10**, silylation of 6-aminopurine-2-sulfonamide [8] (**7**, 2.1 g, 10 mmoles) with HMDS (30 ml) and ammonium sulfate (0.1 g), followed by reaction with **8** (3.18 g, 10 mmoles) in the presence of trimethylsilyl triflate (2.78 ml, 14.4 mmoles) in acetonitrile (50 ml) for 5 hours, gave the crude reaction product. The product was purified by flash chromatography over silica gel (3 x 20 cm) using dichloromethane:methanol (98:2, 96:4, v/v) as the eluent to yield 2.5 g (53%) of **11** as white foam; ir: ν max 1040 (S=O), 1230, 1350 (O=S=O), 1745 (C=O of acetyls), 3000-3500 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 261 nm (ε 10,500); (*p*H 7) 260 nm (ε 10,900); (*p*H 11) 260 nm (ε 12,400); ^1H nmr (DMSO-d_6): δ 1.99, 2.05, 2.13 (3 s, 9 H, 3 $COCH_3$), 4.27-4.42 (2 m, 3 H, $C_{4'}H$ and $C_{5'}CH_2$), 5.66 (t, 1 H, $C_{3'}H$), 5.96 (t, 1 H, $C_{4'}H$), 6.24 (d, 1 H, $J_{1',2'}$ = 3.6 Hz, $C_{1'}H$), 7.23 (br s, 2 H, NH_2), 7.99 (br s, 2 H, SO_2NH_2) and 8.57 (s, 1, H, C_8H).

Anal. Calcd. for $C_{16}H_{20}N_6O_9S$ (472.41): C, 40.68; H, 4.27; N, 17.79; S, 6.68. Found: C, 40.93; H, 4.48; N, 17.54; S, 6.60.

6-Amino-9-β-*D*-ribofuranosylpurine-2-sulfonamide (2-Sulfamoyladenosine, **4**). Method A.

A solution of **11** (1.18 g, 2.5 mmoles) in ethanolic ammonia (80 ml, saturated at 0°) was stored at 4° for 15 hours, and then evaporated to dryness. The residue was dissolved in methanol (10 ml), adsorbed onto silica gel (5 g) and purified by flash chromatography over silica gel column (1.5 x 20 cm) packed in dichloromethane. The column was eluted successively with 200 ml portions of dichloromethane:methanol (85:15, 80:20, v/v). The homogeneous fractions were pooled and evaporated. The residue was crystallized from methanol to yield 0.7 g (80%) of **4** as needles, mp 230° dec; ir: ν max 1150 (S=O), 1330 (O=S=O), 3000-3550 (NH_2) cm^{-1}; uv: λ max (*p*H 1) 261 nm (ε 10,900); (*p*H 7) 261 nm (ε 11,300); (*p*H 11) 260 nm (ε 12, 900); ^1H nmr (DMSO-d_6): δ 3.61 (m, 2 H, $C_{5'}CH_2$), 3.93 (d, 1 H, $C_{4'}H$), 4.13 (m, 1 H, $C_{3'}H$), 4.60 (m, 1H, $C_{2'}H$), 5.01 (t, 1 H, $C_{5'}OH$), 5.25 (d, 1 H, $C_{3'}OH$), 5.50 (d, 1 H, $C_{2'}OH$), 5.90 (d, 1 H, $J_{1',2'}$ = 6.1 Hz, $C_{1'}H$), 7.26 (br s, 2 H, NH_2), 7.86 (br s, 2 H, SO_2NH_2) and 8.55 (s, 1 H, C_8H).

Anal. Calcd. for $C_{10}H_{14}N_6O_6S$ (346.32): C, 34.68; H, 4.07; N, 24.27; S, 9.26. Found: C, 34.67; H, 4.03; N, 23.95; S, 9.01.

Method B.

Compound **9** (0.25 g, 0.5 mmole) was combined with liquid ammonia (10 ml) in a steel reaction vessel (25 ml), and heated at 100° for 6 hours. The reaction vessel was cooled, opened carefully and the ammonia was allowed to evaporate at room temperature. The residue was dissolved in methanol (10 ml), filtered and the filtrate was adsorbed onto silica gel (5 g) and placed on top of a silica gel column (1.5 x 20 cm) packed in dichloromethane. The column was eluted with dichloromethane:methanol (8:2, v/v). The homogeneous fractions were pooled and evaporated to dryness. The residue was crystallized from methanol to yield 0.1 g (58%) of **4**, mp 230° dec, and was found to be identical in all respects (tlc, ir, uv and ^1H nmr) to **4** prepared by Method A.

Method C.

A solution of **10** (0.25 g, 0.5 mmole) in ethanolic ammonia (20 ml, saturated at 0°) was stored at 0° for 15 hours, and then evaporated to dryness. The residue was purified as described in Method A to yield 90 mg (52%) of **4**, mp 229-230° dec. This product was found to be identical to **4** prepared by Method A.

Method D.

To a stirred and ice-cooled (0-5°) solution of **13** (0.31 g, 1 mmole) in ethanol (50 ml) was added during 10 minutes a solution of commercial *m*-chloroperoxybenzoic acid (MCPBA, 80-85%, 0.84 g, 4 mmoles) in ethanol (25 ml). After stirring for 10 hours at room temperature, the solvent was evaporated to dryness. A solution of the residue in methanol (10 ml) was adsorbed onto silica gel (5 g) and purified by flash chromatography as described in Method A to yield 0.2 g (58%) of the title compound, mp 229-230° dec. This product was found to be identical to 2-sulfamoyladenosine (**4**) prepared by Method A.

6-Amino-9-β-D-ribofuranosylpurine-2-sulfenamide (**13**).

Commercial 0.77 *M* sodium hypochlorite solution (chlorox, 5.25%, 1.5 ml) was cooled to 0° in an ice bath. Ammonium hydroxide (0.77 M, 0.4 ml) was similarly cooled in an ice bath and added with stirring to the bleach solution. The mixture was stirred at 0° for 15 minutes and then a cold (0°) solution of 6-amino-9-β-D-ribofuranosylpurine-2(3*H*)-thione [14,15] (2-thioadenosine, **12**, 0.3 g, 1 mmole) in 2 *N* potassium hydroxide solution (0.5 ml) was added. The flask was stoppered and stirred for 2 hours and then allowed to slowly warm to room temperature. The solvents were evaporated to dryness. The residue was dissolved in methanol (5 ml) and adsorbed onto silica gel (5 g). The excess solvent was evaporated and the dry residue was loaded onto a silica gel column (1.5 x 20 cm) packed in dichloromethane. The column was eluted with a mixture of dichloromethane:methanol (8:2, 7:3, v/v). The homogeneous fractions were pooled, the solvents evaporated and the residue was crystallized from aqueous methanol to yield 0.16 g (51%) of **13**, mp >100° dec; ir: ν max 3100-3450 (NH_2, OH) cm^{-1}; uv: λ max (*p*H 1) 270 nm (ε 7,300); (*p*H 7) 274 nm (ε 7,600); (*p*H 11) 274 nm (ε 8,100); ^1H nmr (DMSO-d_6): δ 3.59 (m, 2 H, $C_{5'}CH_2$), 3.84 (s, 2 H, SNH_2), 3.91 (m, 1 H, $C_{4'}H$), 4.14 (m, 1 H, $C_{3'}H$), 4.60 (t, 1 H, $C_{2'}H$), 5.08 (t, 1 H, $C_{5'}OH$), 5.22 (br s, 1 H, $C_{3'}OH$), 5.45 (br s, 1 H, $C_{2'}OH$), 5.86 (d, 1 H, $J_{1',2'}$ = 4.1 Hz, $C_{1'}H$), 7.36 (s, 2 H, NH_2) and 8.21 (s, 1 H, C_8H).

Anal. Calcd. for $C_{10}H_{14}N_6O_4S$ (314.32): C, 38.21; H, 4.49; N, 26.74; S, 10.20. Found: C, 38.44; H, 4.44; N, 26.94; S, 9.92.

X-ray Structure Determination of 2-Sulfamoyladenosine (**4**).

Colorless, transparent, diamond-shaped crystals of **4** grew from a methanol:dichloromethane (9:1, v/v) solution by slow evaporation. A summary of crystal data and refinement is given in Table 4. Positions of all non-hydrogen atoms were determined by direct methods [19]. The disordered 05'D (1.33 e/Å^3) as well as all hydrogen atom positions were observed in a difference electron density map calculated at R = 0.046 (peaks were 0.43-0.87 e/Å^3). The C5'-05' and C5'-05'D distances were constrained to be equal. The disorder was treated by idealizing the H5' atoms with respect to the two 05' positions such that all C5'-H5' distances were 1.0

Å and the H5'-C5'-H5' angles were 109.5° for each 05' position. All C4'-H5' distances were constrained to be equal; likewise, all of the 05'-H5' and 05'D-H5' distances were constrained to be equal. Parameters refined by full-matrix least-squares [20] include the following: all atomic positions within the bounds of the above constraints; anisotropic thermal parameters for all non-hydrogen atoms except 05'D which was treated isotropically; individual isotropic thermal parameters except for the H5' set which had a common isotropic thermal parameter; and, the site occupation for the 05', 05'D and pertinent hydrogen atoms (set of H5' and H05') which refined to 0.86:0.14 for 05':05'D. Atomic scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from International Tables for X-ray Crystallography [21]. For hydrogen, these parameters were taken from Stewart, Davidson and Simpson [22]. Figures were drawn with ORTEPII [23]; the least-squares planes program was obtained from Cordes [24].

REFERENCES AND NOTES

[†] Present address: Triplex Pharmaceutical Corporation, 9391 Grogan's Mill Road, The Woodlands, Texas 77380.

[1] G. B. Elion, S. Bieber and G. H. Hitchings, *Ann. N.Y. Acad. Sci.*, **60**, 297 (1954).

[2] J. H. Burchenal, M. L. Murphy, R. R. Ellison, M. P. Sykes, C. T. C. Tan, L. A. Leone, D. A. Karnofsky, L. F. Craver, H. W. Dargeon and C. P. Rhoads, *Blood*, **8**, 965 (1953).

[3] R. R. Ellison and J. H. Burchenal, *Clin. Pharm. Ther.*, **1**, 631 (1960).

[4] S. Herber, L. Lennard, J. S. Lilleyman and J. Maddocks, *Brit. J. Cancer*, **46**, 138 (1982).

[5] J. O. Liliemark, M. Masquelier and C. O. Peterson, *Proc. Am. Assoc. Cancer Res.*, **79**, 350 (1988).

[6] S. Matsumura, T. Hoshino, M. Weizaecker and D. F. Dean, *Cancer Treat. Rep.*, **67**, 475 (1983).

[7] J. V. Simone, *Brit. J. Haematol.*, **45**, 1 (1980).

[8] A. G. Beaman and R. K. Robins, *J. Am. Chem. Soc.*, **83**, 4038 (1961).

[9] L. R. Lewis, C. W. Noell, A. G. Beaman and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 607 (1962).

[10] R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964).

[11] R. K. Robins and G. R. Revankar, *Med. Res. Rev.*, **5**, 273 (1985).

[12] G. R. Revankar, N. B. Hanna, N. Imamura, A. F. Lewis, S. B. Larson, R. A. Finch, T. L. Avery and R. K. Robins, *J. Med. Chem.*, **33**, 121 (1990).

[13] H. Vorbrüggen, K. Krolikiewiez and B. Bennua, *Chem. Ber.*, **114**, 1234 (1981).

[14] K. Kikugawa, H. Suehiro and M. Ichino, *J. Med. Chem.*, **16**, 1381 (1973).

[15] K. Kikugawa and J. Suehiro, *J. Carbohydr. Nucleosides Nucleotides*, **2**, 159 (1975).

[16] T. F. Lai and R. E. Marsh, *Acta Cryst.*, **B28**, 1982 (1972).

[17] C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).

[18] For experimental details, see: M. Kuehl, D. I. Brixner, A. D. Broom, T. L. Avery and R. L. Blakley, *Cancer Res.*, **48**, 1481 (1988).

[19] G. M. Sheldrick, SHELXS86. Program for Crystal Structure Determination, University of Göttingen, Federal Republic of Germany, 1986.

[20] G. M. Sheldrick, SHELX76. Program for Crystal Structure Determination, University of Cambridge, England, 1976.

[21] International Tables for X-ray Crystallography, Vol **IV**, J. A. Ibers and W. C. Hamilton, eds, Kynoch Press: Birmingham, England, 1974, pp 99, 149.

[22] R. F. Stewart, E. R. Davidson and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).

[23] C. K. Johnson, OR TEPII. A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations, Oak Ridge National Laboratory ORNL-5138 Third Revision, March, 1976.

[24] A. W. Cordes, Personal Communication (1983).