Inhibitors of Nucleoside Transport. A Structure–Activity Study Using Human Erythrocytes†

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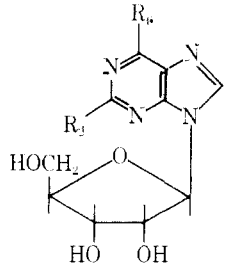
The passage of nucleosides across the plasma membrane of erythrocytes is a membrane-mediated process which is strongly inhibited by derivatives of 9-β-D-ribofuranosylpurine (1) with S, O, or N atoms at the purine 6 position bearing variously substituted arylalkyl groups. In this structure–activity study, nucleoside derivatives were compared in respect to their ability to inhibit a transport-dependent aspect of nucleoside metabolism in erythrocytes, the synthesis of inosine from external guanosine and hypoxanthine. 6-Benzylthio, 6-benzylamino, and 6-benzyloxy derivatives of 1 were inhibitory at 10^{-5}–10^{-6} *M* and the similarity of their activities suggested that alkylation of the transporter as the mechanism of transport inhibition was unlikely. The hydrophobicity of the 6-position substituents appeared to contribute importantly to inhibitory activity. Although replacement of the ribofuranose moiety by other sugars reduced inhibitory activity, compounds with 9-butyl groups were inhibitory. 6-[(2-Hydroxy-5-nitrobenzyl)thio] derivatives of 1 were the most potent of the inhibitors tested, being active at about 10^{-7} *M*.

Nucleoside permeation in erythrocytes[1-4] and in various other cell types[5-9] has been recognized as a process mediated by transport elements of the plasma membrane. Studies in this laboratory have demonstrated that uridine transport by human erythrocytes is a "facilitated diffusion" process.[1,3,4] The uridine transport mechanism of the erythrocyte is of broad specificity in that it accepts as "substrates" ribosyl and deoxyribosyl derivatives of both purines and pyrimidines.[1,3,4]

A number of compounds are potent inhibitors of nucleoside transport, including persantin,[10-12] cytochalasin B,[13] and colchicine;[9] in this laboratory various purine nucleoside derivatives were found to be potent and specific inhibitors of nucleoside transport in erythrocytes and other cells.[3,4,14-18] Of these, S-substituted derivatives of 6-thioinosine and 6-thioguanosine were particularly potent; for example, S^6-(4-nitrobenzyl)thioguanosine (2) at concentra-



compd	R_2	R_6
1	H	H
2	NH_2	4-nitrobenzylthio
3	H	4-nitrobenzylthio
4	NH_2	H

tions in the order of 10^{-5} *M* totally blocked both influx and efflux of thymidine and uridine,[3,4] and S^6-(4-nitrobenzyl)thioinosine (3) was bound firmly, but reversibly to the erythrocyte membrane. The uptake of 3 by erythrocytes had two components, one of which was directly proportional to the external concentration of 3 and, thus, was likely due to absorptive and diffusional uptake.[16] The other component of 3 uptake occurred through the reversible binding of 3 to specific sites in the erythrocyte membrane; each erythrocyte has approximately 10^4 such sites to which 3 binds with an association constant of 10^9 *M*.[17] Evidence has been presented that the binding sites for 3 are the uridine transport elements of the membrane,[17] which become inactivated when 3 and related compounds are bound.

In the present study, a group of nucleoside derivatives was assayed for inhibition of a transport-dependent aspect of nucleoside metabolism in erythrocytes, the conversion of externally provided guanosine and hypoxanthine to inosine. When washed, intact erythrocytes are incubated in medium containing hypoxanthine and guanosine, these substrates enter the cells and are metabolized in a two-step process catalyzed by purine nucleoside phosphorylase, and the inosine product is released into the incubation medium:

guanosine + phosphate ↔ guanine + ribose 1-phosphate

[^{14}C]hypoxanthine + ribose 1-phosphate ↔ [^{14}C]inosine + phosphate

sum: [^{14}C]hypoxanthine + guanosine ↔ [^{14}C]inosine + guanine

Inosine synthesis does not proceed in the absence of extracellular guanosine. The rate at which inosine appears in the extracellular medium is a measure of nucleoside transport because (a) inosine synthesis is blocked by 3, which is known to interact with the nucleoside transport system of human erythrocytes,[17] and (b) this reaction, when proceeding in preparations of disrupted erythrocytes, is not affected by 3 and related compounds.[19]

Results and Discussion

Nucleoside derivatives were assayed at various concentrations for inhibition of inosine synthesis and observed rates (μmol/g of cells/15 min) were expressed as percentages of inosine formation in control incubation mixtures which contained no test compound. Concentrations of test compounds which inhibited control rates of inosine synthesis by 50% (IC_{50}, m*M*) were determined.

Table I is a compilation of IC_{50} values for various nucleoside derivatives. From these data, inferences can be made about chemical features of the inhibitor molecules responsible for interaction with the nucleoside transport mechanism. In the discussion which follows, data from Table I are grouped in smaller tables to illustrate certain structure–activity relationships.

Table II demonstrates that derivatives of 1 with S, O, or N atoms at the purine 6 position bearing arylalkyl substituents were potent inhibitors of transport. The 6-benzylthio, 6-benzyloxy, and 6-benzylamino derivatives of 1 had comparable activity and, accordingly, it is unlikely that the inhibitor–cell interaction involved alkylation of the nucleoside transport sites. This conclusion is supported by evidence that covalent linkages are not involved in the binding of 3 to the erythrocyte membrane.[16,17] The 6-sulfur, oxygen, and nitrogen atoms may contribute to inhibitor binding through hydrogen bonding.

The data of Table III suggest (a) that 6-(RCH_2S) derivatives of 1 and of 2-amino-9-β-D-ribofuranosylpurine (4) are more potent inhibitors than analogous compounds in which

†Parts of this work were presented at the 166th National Meeting of the American Chemical Society, Chicago, Ill., August 1973, Abstracts, BIOL 239.

Table I. Inhibitors of Nucleoside Transport in Human Erythrocytes

Compd		Source[a]	IC_{50},[b] m*M*
	Adenosine Derivatives		
5	N^6-*n*-Amyladenosine	E	2.8×10^{-2}
6	N^6-Benzyladenosine	D, E	2.2×10^{-2}
7	N^6-2-Ethoxyethyladenosine	E	1.9
8	N^6-Furfuryladenosine	E	7.6×10^{-2}
9	N^6-Isopropyladenosine	E	5.4×10^{-1}
10	N^6-Methyl-N^6-(4-nitrobenzyl)adenosine	D	6.4×10^{-4}
11	N^6-(4-Nitrobenzyl)-adenosine	D	5.8×10^{-5}
12	N^6-Phenyladenosine	E	6.8×10^{-2}
13	N^6-2-Thenyladenosine	E	3.7×10^{-2}
	Inosine Derivatives		
14	O^6-Benzylinosine	D	4.4×10^{-3}
	6-Mercaptopurine Derivatives		
15	6-(Allylthio)-9-β-D-ribofuranosylpurine	A	2.9×10^{-2}
16	6-(Benzylthio)-9-β-D-ribofuranosylpurine	A	3.5×10^{-3}
17	6-(Cyclohexylmethylthio)-9-β-D-ribofuranosylpurine	D	3.2×10^{-3}
18	6-(Cyclohexylthio)-9-β-D-ribofuranosylpurine	D	1.3×10^{-2}
19	6-(α, α-Dimethylbenzylthio)-9-β-D-ribofuranosylpurine	D	4.5×10^{-2}
20	6-(Ethylthio)-9-β-D-ribofuranosylpurine	B	1.2×10^{-1}
21	6-[(2-Hydroxy-5-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine	D	6.9×10^{-5}
22	6-[(4-Isopropylbenzyl)thio]-9-β-D-ribofuranosylpurine	E	1×10^{-2}
23	6-[(2-Methyl-1-naphthyl)-methylthio]-9-β-D-ribofuranosylpurine	A	2.4×10^{-1}
24	6-(Methylthio)-9-β-D-ribofuranosylpurine	A	4.8×10^{-1}
25	6-(Methylthio)-9-(tetrahydropyran-2-yl)purine	A	5.8×10^{-1}
3	6-[(4-Nitrobenzyl)thio]-9-β-D-ribofuranosylpurine	D	1.5×10^{-5}
26	6-(Phenylpropylthio)-9-β-D-ribofuranosylpurine	D	2.8×10^{-3}
27	6-(Phenylthio)-9-β-D-ribofuranosylpurine	D	1.9×10^{-1}
28	6-(2-Methylbenzylthio)-9-β-D-ribofuranosylpurine	D	1.3×10^{-2}
29	6-(3-Methylbenzylthio)-9-β-D-ribofuranosylpurine	D	2.5×10^{-2}
30	6-(4-Methylbenzylthio)-9-β-D-ribofuranosylpurine	D	9.6×10^{-4}
	6-Thioguanine Derivatives		
31	2-Amino-6-(benzylthio)-9-β-D-ribofuranosylpurine	A	1.1×10^{-2}
32	2-Amino-6-[(3-bromobenzyl)thio]-9-β-D-ribofuranosylpurine	C	6.0×10^{-3}
33	2-Amino-6-[(4-bromobenzyl)thio]-9-β-D-ribofuranosylpurine	C	1.7×10^{-3}
34	2-Amino-9-butyl-6-(isopropylthio)purine	C	2.5×10^{-1}
35	2-Amino-9-butyl-6-(methylthio)purine	A	4.6×10^{-1}
36	2-Amino-9-butyl-6-[(2-pyridylmethyl)thio]purine	C	1.1×10^{-1}
37	2-Amino-6-(butylthio)-9-β-D-ribofuranosylpurine	C	2.5×10^{-2}
38	2-Amino-6-(*sec*-butylthio)-9-β-D-ribofuranosylpurine	C	1.3×10^{-1}
39	2-Amino-6-[(2-chlorobenzyl)thio]-9-β-D-ribofuranosylpurine	C	2.8×10^{-2}
40	2-Amino-6-(ethylthio)-9-β-D-ribofuranosylpurine	A	3.4×10^{-1}
41	2-Amino-6-[(2-fluorobenzyl)thio]-9-β-D-ribofuranosylpurine	C	1.0×10^{-2}
42	2-Amino-6-[(4-fluorobenzyl)thio]-9-β-D-ribofuranosylpurine	A	2.4×10^{-3}
43	2-Amino-6-[(2-hydroxy-5-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine	D	5.8×10^{-6}
44	2-Amino-6-iodo-9-β-D-ribofuranosylpurine	C	1.7×10^{-1}
45	2-Amino-9-isobutyl-6-propylthiopurine	C	1.3×10^{-1}
46	2-Amino-6-(isobutylthio)-9-β-D-ribofuranosylpurine	A	1.8×10^{-2}
47	2-Amino-6-(isopropylthio)-9-propylpurine	C	3×10^{-1}
48	2-Amino-6-(isopropylthio)-9-β-D-ribofuranosylpurine	A	4.0×10^{-1}
49	2-Amino-9-(2-methylbutyl)-6-[(2-pyridylmethyl)thio]-purine	C	8.6×10^{-2}
50	2-Amino-6-[(1-methyl-4-nitroimidazol-5-yl)thio]-9-β-D-ribofuranosylpurine	A	1.2
51	2-Amino-6-[(6-methyl-2-pyridylmethyl)thio]-9-β-D-ribofuranosylpurine	A	2.4×10
52	2-Amino-6-(methylthio)-9-β-D-ribofuranosylpurine	A	4.3×10^{-1}
53	2-Amino-6-[(2-nitrobenzyl)-thio]-9-β-D-ribofuranosylpurine	A	9.6×10^{-3}
54	2-Amino-6-[(3-nitrobenzyl)-thio]-9-β-D-ribofuranosylpurine	D	3.7×10^{-4}
2	2-Amino-6-[(4-nitrobenzyl)-thio]-9-β-D-ribofuranosylpurine	A	4.5×10^{-5}
55	2-Amino-6-(phenethylthio)-9-β-D-ribofuranosylpurine	A	3.4×10^{-2}
56	2-Amino-9-propyl-6-(2-pyridylmethylthio)purine	C	1.9×10^{-1}
57	2-Amino-6-(propylthio)-9-β-D-ribofuranosylpurine	A	6.7×10^{-2}
58	2-Amino-6-[(2-pyridylmethyl)thio]-9-β-D-ribofuranosylpurine	C	7.2×10^{-2}
59	2-Amino-6-[(3-pyridylmethyl)thio]-9-β-D-ribofuranosylpurine	C	3.1×10^{-2}

Table I (*Continued*)

Compd		Source[a]	IC_{50},[b] m*M*
60	2-(2-Amino-9-β-D-ribofuranosylpurine-6-ylthio)acetophenone	A	9.4×10^{-1}
61	2-(2-Amino-9-β-D-ribofuranosylpurin-6-ylthio)-4′-chloroacetophenone	A	3.0×10^{-2}
	Pyrazolo[3,4-*d*]pyrimidine Derivatives		
62	4-Isopentylaminopyrazolo[3,4-*d*]pyrimidine	A	4.9×10^{-1}
63	4-Phenethylaminopyrazolo[3,4-*d*]pyrimidine	A	2.1×10^{-1}
	Pyrrolo[2,3-*d*]pyrimidine Derivatives		
64	4-Amino-5-iodo-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine	A	4.8×10^{-1}
65	4-(Benzylthio)-7-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyrimidine	A	1.1×10^{-2}
66	5-Bromo-4-(methylthio)-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine	A	2.4×10^{-1}
67	4-Chloro-5-iodo-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine	A	1.6×10^{-1}
68	4-Methoxy-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine	A	7.1×10^{-1}
69	4-Piperidino-7-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyrimidine	A	4.1×10^{-1}
70	7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine-4-thiol	A	8.1×10^{-1}

[a] See Experimental Section. [b] Concentration (m*M*) of test compound that reduced the rate of inosine synthesis to 50% of that in control incubation mixtures containing no inhibitor.

Table II. Inhibitors of Nucleoside Transport. Substituents at the 6 Position of Ribofuranosylpurine (1) and 2-Aminoribofuranosylpurine (4)

Compd	Derivative of	6-Substituent	IC_{50},[a] m*M*
31	4	S-Benzyl	1.1×10^{-2}
16	1	S-Benzyl	3.5×10^{-4}
14	1	O-Benzyl	4.4×10^{-3}
6	1	HN-Benzyl	2.2×10^{-2}
2	4	S-(4-Nitrobenzyl)	4.5×10^{-5}
3	1	S-(4-Nitrobenzyl)	1.5×10^{-5}
11	1	HN-(4-Nitrobenzyl)	5.8×10^{-5}
10	1	(CH_3)N-(4-Nitrobenzyl)	6.4×10^{-4}

[a] See Table I.

Table III. Inhibitors of Nucleoside Transport. 6-(R′-Thio) Derivatives of Ribofuranosylpurine (1) and 2-Aminoribofuranosylpurine (4)

Compd	R′	IC_{50},[a] m*M*
Thioguanosine Derivatives [6-(R′-Thio) Derivatives of 4]		
52	Methyl	4.3×10^{-1}
40	Ethyl	3.4×10^{-1}
57	Propyl	6.7×10^{-2}
48	Isopropyl	4.0×10^{-1}
37	*n*-Butyl	2.5×10^{-2}
38	*sec*-Butyl	1.3×10^{-1}
46	Isobutyl	1.8×10^{-2}
31	Benzyl	1.1×10^{-2}
55	Phenylethyl	3.4×10^{-2}
Thioinosine Derivatives [6-(R′-Thio) Derivatives of 1]		
20	Ethyl	1.2×10^{-1}
15	Allyl	2.9×10^{-2}
16	Benzyl	3.5×10^{-4}
17	Cyclohexylmethyl	3.2×10^{-4}
27	Phenyl	1.9×10^{-1}
18	Cyclohexyl	1.3×10^{-2}
26	Phenylpropyl	2.8×10^{-4}
23	2-Methyl-1-naphthylmethyl	2.4×10^{-1}
19	α,α-Dimethylbenzyl	4.5×10^{-2}

[a] See Table I.

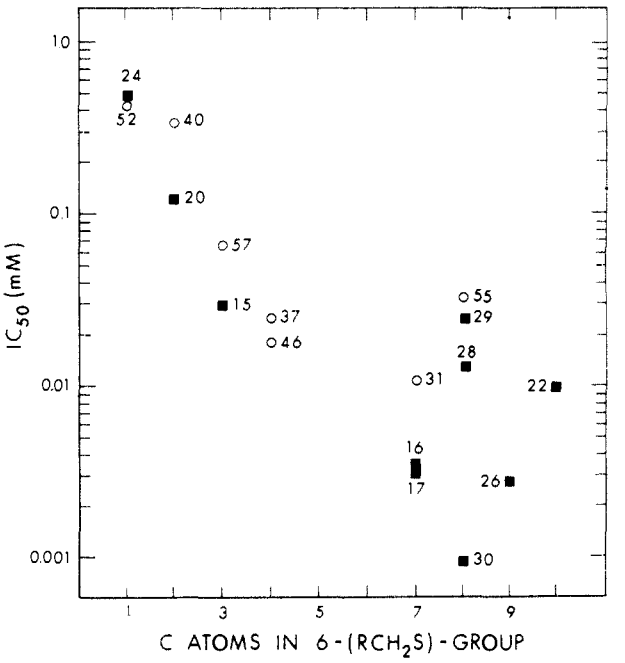


Figure 1. Inhibitors of nucleoside transport. 6-(RCH_2S) derivatives of 1 and 4. IC_{50} values from Table I are plotted against the number of carbon atoms in the 6-(RCH_2S) substituent group for derivatives of 1 (■) and 4 (○). Compound numbers from Table I are given.

the carbon atom nearest to the sulfur has additional C–C bonds (e.g., compound **19**, Table I), and (b) that inhibitory activity of the 6-(RCH_2S) derivatives is related to the number of carbon atoms in the substituent, within bulk tolerance limits (evidently exceeded in the case of compound **23**). The latter relationship is illustrated in Figure 1 in which IC_{50} values of 1 and 4 derivatives from Table I are plotted against the number of carbon atoms in the 6-(RCH_2S) substituent, where R is a hydrocarbon group. These data suggest that (a) hydrophobic bonding through the S substituent contributed to the inhibitor–cell interaction, and (b) an unsubstituted *S*-methylene link allowed this interaction to take place. Because benzyl and cyclohexylmethylthio derivatives had similar inhibitory activity, it would seem that π–π electron interaction between the inhibitor molecule and the binding site(s) on the transporter does not occur.

The inhibitory activities of the 6-benzylthio derivatives

Table IV. Inhibitors of Nucleoside Transport. 6-[(R-Benzyl)thio] Derivatives of Ribofuranosylpurine (1) and 2-Aminoribofuranosylpurine (4)

Compd	Benzyl substituent (R)	IC_{50},[a] m*M*
	Derivatives of 1	
16	None	3.5×10^{-3}
28	2-Methyl	1.3×10^{-2}
29	3-Methyl	2.5×10^{-2}
30	4-Methyl	9.6×10^{-4}
	Derivatives of 4	
31	None	1.1×10^{-2}
53	2-Nitro	9.6×10^{-3}
54	3-Nitro	3.7×10^{-4}
2	4-Nitro	4.5×10^{-5}

[a] See Table I.

of **1** and **4** were markedly enhanced by the presence of a nitro group at the benzyl 4 position (Table II); the 6-[(2-hydroxy-5-nitrobenzyl)thio] derivatives of **1** and **4** (compounds **21** and **43**) were very potent inhibitors, having IC_{50} values, respectively, 0.01 and 0.001 times those of the corresponding derivatives with unsubstituted benzyl groups (compounds **16** and **31**). The inhibitory activity of 6-(benzylthio)-**4** (compound **31**) was not appreciably changed by the addition of a nitro group at the benzyl 2 position (**53**), whereas the 3-nitrobenzyl derivative (**54**) was a more effective inhibitor, the IC_{50} being 0.03 that of **31**. The presence of halogen atoms on the 6-benzylthio substituent of **31** did not markedly change the inhibitory activity. Charge-transfer complex[20,21] formation between the electrophilic nitro substituent and a nucleophilic group at the periphery of the hydrophobic region of the carrier binding site could be involved in the large increases in the apparent affinity of the inhibitor for cellular binding sites arising from the presence of 3- and 4-nitro groups on the benzyl substituent. However, it is apparent in Table IV that 4-methyl and 4-nitrobenzylthio derivatives of **1** and **4** are more potent inhibitors than the corresponding 2- or 3-substituted derivatives and, thus, steric considerations are also important in this aspect of the inhibitor–cell interaction. Nitro substituents on the benzyl group may participate in hydrophobic binding.[22]

It is noted that the 6-iodo derivative of **4** (compound **44**) showed inhibitory activity comparable to the small alkylthio derivatives; such activity was also reported earlier.[19,23]

Table I includes various pairs of nucleosides which differ only in respect to the absence or presence of an amino group at the purine 2 position (compounds **24, 52; 16, 31; 3, 2; 21, 43**); it is apparent that the 2-amino group does not have a substantial influence on the inhibitory activity of the S^6 derivatives.

Derivatives of the purine analogs, pyrazolo[3,4-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine, showed only moderate inhibitory activity (Table I). Compound **65**, the 7-deaza homolog of 6-benzylthioinosine (**16**), was a much less effective inhibitor than the latter, suggesting that the purine 7-nitrogen is involved in the inhibitor–cell interaction. Two other pyrrolo[2,3-*d*]pyrimidine ribonucleosides, compounds **68** and **70**, were only slightly more inhibitory than the corresponding purine homologs tested previously.[15,19]

In previous reports, it was apparent that 9-alkyl or 9-β-D-ribofuranosyl derivatives of purines having 6-position substituents were inhibitory in this system, but purine derivatives which lacked 9-substituents were inactive.[19,23] In the present study, various 9-alkyl substituents (in particular, the butyl group) imparted inhibitory activity comparable to that found for the corresponding ribosyl derivatives (Table V). Accordingly, it would appear that there exists on the nucleoside carrier a hydrophobic region which is part of, or adjacent to, the ribosyl binding site; this situation may be analogous to the hydrophobic region of adenosine deaminase which has been recognized by Schaeffer and Schwender[24] as being involved in the binding of the 9-position substituents of some 9-(2-hydroxy-3-alkyl)adenines which are very potent inhibitors of adenosine deaminase.

In summary, purine ribonucleosides with S, O, or N atoms at the purine 6 position bearing alkyl or variously substituted arylalkyl groups are potent inhibitors of nucleoside transport in human red cells. The 6-position substitu-

Table V. Inhibitors of Nucleoside Transport. 2-Amino-6-(R-thio)-9-R′-purines

Compd	R	R′	IC_{50},[a] m*M*
52	Methyl	Ribofuranosyl	4.3×10^{-1}
35	Methyl	Butyl	4.6×10^{-1}
48	Isopropyl	Ribofuranosyl	4.0×10^{-1}
34	Isopropyl	Butyl	2.5×10^{-1}
47	Isopropyl	Propyl	3×10^{-1}
57	Propyl	Ribofuranosyl	6.7×10^{-2}
45	Propyl	Isobutyl	1.3×10^{-1}
58	2-Pyridylmethyl	Ribofuranosyl	7.2×10^{-2}
36	2-Pyridylmethyl	Butyl	1.1×10^{-1}
49	2-Pyridylmethyl	2-Methylbutyl	8.6×10^{-2}
56	2-Pyridylmethyl	Propyl	1.9×10^{-1}

[a] See Table I.

Table VI. Preparation of Purine Nucleosides

Compd	Group on N^6, O^6, or S^6	Mp, °C	Yield, %	Crystn solvent
	N^6 Derivatives of Adenosine			
6	Benzyl[a]	171	84	MeOH
10	N^6-Methyl-N^6-(4-nitrobenzyl)	151	80	MeOH–Et_2O
11	4-Nitrobenzyl	176	70	MeOH
	O^6 Derivatives of Inosine			
14	Benzyl	173	91	EtOAc–petr ether
	S^6 Derivatives of 6-Thioinosine			
3	4-Nitrobenzyl	198	68	EtOAc
17	Cyclohexylmethyl	101	87	EtOAc–petr ether
18	Cyclohexyl	165	82	EtOAc–petr ether
19	α,α-Dimethylbenzyl	126	75	EtOAc–petr ether
21	2-Hydroxy-5-nitrobenzyl	156	81	EtOAc
26	Phenylpropyl	159	78	EtOAc–heptane
27	Phenyl	202	72	Et_2O
28	2-Methylbenzyl	189	86	EtOAc
29	3-Methylbenzyl	176	88	EtOAc
30	4-Methylbenzyl	100	88	EtOAc–petr ether
	S^6 Derivatives of 6-Thioguanosine			
43	2-Hydroxy-5-nitrobenzyl	184	83	EtOAc
54	3-Nitrobenzyl	125	76	Me_2CO

[a] See ref 29.

Table VII. R_f Values in Paper Chromatography

Compd	R_f in these solvent systems[a]							
	A	B	C	D	E	F	G	H
3		0.85	0.88	0.79		0.80	0.83	
6	0.73	0.82	0.84	0.82	0.69	0.82	0.68	
10	0.84	0.84	0.85	0.78	0.99	0.81	0.67	0.45
11	0.77	0.79	0.83	0.86	0.63	0.79	0.68	0.42
14	0.77	0.83	0.84	0.25	0.71	0.83	0.70	0.52
17	0.86	0.85	0.90	0.97	0.82	0.83	0.83	
18	0.86	0.85	0.87	0.99	0.81	0.85	0.82	0.29
19	0.91	0.87	0.87	0.31	0.84	0.87	0.93	0.65
21	0.87	0.71	0.87	0.83	0.16	0.79	0.83	
26	0.87	0.84	0.89	0.97	0.81	0.85	0.93	
27	0.76	0.80	0.82	0.80	0.69	0.83	0.66	
28	0.90	0.83	0.88	0.97	0.76	0.83	0.92	
29	0.85	0.86	0.89	0.96	0.79	0.84	0.89	
30	0.85	0.86	0.87	0.96	0.74	0.83	0.76	
43	0.80	0.58	0.79	0.72	0.17	0.75	0.79	
54	0.80	0.75	0.80	0.57	0.79	0.69		

[a]Solvent systems used for descending chromatography on Whatman No. 1 paper were A, EtOAc-*n*-PrOH-H_2O (4:1:2); B, *i*-PrOH-concentrated NH_4OH-H_2O (70:5:25); C, *i*-PrOH-1% $(NH_4)_2SO_4$ (2:1); D, *i*-PrOH-concentrated HCl-H_2O (680:170:144); E, *n*-BuOH-H_2O-concentrated NH_4OH (86:14:5); F, *n*-BuOH-AcOH-H_2O (5:2:3); G, H_2O-saturated BuOH; H, 0.1 *M* potassium phosphate, pH 6.7.

Table VIII. Spectral Data

Compd	0.1 *N* HCl		H_2O		0.1 *N* NaOH	
	λ_{max}[a]	$\epsilon \times 10^{-4}$[b]	λ_{max}	$\epsilon \times 10^{-4}$	λ_{max}	$\epsilon \times 10^{-4}$
3	291	2.44	290	2.51	289	2.36
6	265	2.05	269	2.09	270	2.14
10	278	2.64	278	2.76	278	2.81
11	273	2.44	271	2.55	271	2.55
14	251	1.26	251	1.34	251	1.34
17	296	1.99	294	1.96	294	2.04
18	296	1.75	294	2.12	294	1.95
19	323	2.14	293	1.41	293	1.42
21	293	2.29	292	2.32	293	2.05
26	296	1.80	294	1.92	294	1.92
27	290	2.14	290	2.00	290	2.14
28	294	1.98	294	2.10	294	2.10
29	294	1.98	294	2.10	294	2.10
30	295	2.00	294	2.07	294	2.07
43	320	1.99	314	1.90	312	1.46
54	318	1.31	312	1.47	312	1.42

[a]nm. [b]Molar absorbance.

ents appear to be involved in a hydrophobic interaction with the nucleoside transport mechanism resulting in a firm, but noncovalent binding of the inhibitor. Another hydrophobic binding region on the nucleoside transport mechanism may bind 9-alkyl groups on the inhibitor molecules.

Experimental Section

Nucleoside Transport Assays. Human erythrocytes were obtained from outdated blood provided by the Red Cross Society Blood Transfusion Service, Edmonton. Blood was centrifuged, the supernatant and buffy coat were discarded, and the cells were washed twice in cold 0.154 *M* sodium chloride and suspended in a volume of Krebs Ringer phosphate medium equal to four times the cell mass. Incubation mixtures, prepared in duplicate, contained 50 mg (fresh weight) of erythrocytes, 1.0 μmol each of hypoxanthine-8-^{14}C and guanosine, and test compound in Krebs Ringer phosphate buffer (pH 7.4) in a final volume of 1.0 ml. Incubation mixtures were incubated at 37° for 15 min and, after centrifugation, cell-free medium was heated for 2 min in boiling water; 10-μl portions of medium were chromatographed on paper with "carrier" inosine and hypoxanthine, using 1-butanol-acetic acid–water (5:3:2, v/v). Chromatogram sections were assayed directly for ^{14}C by liquid scintillation methods. The above procedures have been described in detail previously.[19]

Sources of the compounds tested are indicated in Table I as follows: (A) Drug Research and Development, National Cancer Institute, Bethesda, Md.; (B) J. A. Montgomery, Southern Research Institute, Birmingham, Ala.; (C) R. K. Robins, ICN Pharmaceuticals Inc., Irvine, Calif.; (D) this laboratory; (E) M. H. Fleysher, Roswell Park Memorial Institute, Buffalo, N.Y.

Synthetic Methods. 6-(RCH_2S) derivatives of **1** and **4** were made by reacting 6-thioinosine (**71**, 6-thio-9-β-D-ribofuranosylpurine) or 6-thioguanosine (**72**, 2-amino-6-thio-9-β-D-ribofuranosylpurine) with appropriate phenylalkyl halides as described by Montgomery et al.[25] and Noell and Robins[26] (method A). 6-Chloropurine ribonucleoside (**73**, 6-chloro-9-β-D-ribofuranosylpurine) when treated with thiophenol or substituted thiophenols in the presence of sodium methoxide by the method of Johnson et al.[27] gave 6-(phenylthio) derivatives of **1**. N^6-Phenylalkyladenosines were made from **73** by reaction with an appropriate amine by procedures described by Fleysher et al.[28,29] (method B). On heating **73** with benzyl alcohol in the presence of sodium, O^6-benzylinosine was formed (method C).

Microanalyses (C, H, and N) were performed on all the compounds listed (G. I. Robertson, Jr., Florham Park, N.J.) on samples dried at 80° in vacuo over P_2O_5; all analyses agreed with calculated values within ±0.4%. Melting points (uncorrected) were determined on a Kofler micro-hot-stage apparatus; uv spectra were obtained with a Cary Model 15 spectrophotometer and ir spectra in KBr with a Perkin-Elmer 137B spectrometer. 4-Nitrobenzylamine[30] and *N*-methyl-4-nitrobenzylamine[31] were made by reacting 4-nitrobenzyl chloride or bromide with hexamethylenetetraamine and aqueous methylamine (40%), respectively. α,α-Dimethylbenzyl chloride[32] was made by treatment of α,α-dimethylbenzyl alcohol with phosphorus trichloride. Reagent grade dimethylformamide (DMF) was distilled after drying over CaH_2.

Method A. 6-[(4-Nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (3). A mixture of **71** (100 mg, 0.35 mmol), anhydrous potassium carbonate (58 mg, 0.42 mmol), and α-bromo-4-nitrotoluene (91 mg, 0.42 mmol) in 3 ml of dry DMF was heated with stirring at 48–50° for 2 hr. The reaction mixture was cooled and poured into 20 ml of cold H_2O and the solution was adjusted to pH 7 with HCl; the gel-like precipitate was washed with cold H_2O and dried. The filtrate was evaporated in vacuo, the residue extracted with EtOAc (15 ml × 3), and the original precipitate dissolved in the combined extracts; after concentration to 5 ml and cooling to 10°, crystalline material separated out which was washed with cold Et_2O and dried (yield 101 mg (68%), mp 198°). This material gave a single spot in

two TLC systems: MeOH–$CHCl_3$ (1:1) and *n*-BuOH–AcOH–H_2O (6:3:1); R_f values were 0.79 and 0.72, respectively; ir λ_{max}^{KBr} 6.52 (1534) and 7.41 μ (1350 cm^{-1}) (CNO_2).

This method was used to prepare S^6 derivatives of **71** and **72** listed in Table VI.

Method B. N^6-(4-Nitrobenzyl)adenosine (11). A mixture of **73** (200 mg, 0.69 mmol), 4-nitrobenzylamine (320 mg, 2.10 mmol), and calcium carbonate (140 mg, 1.40 mmol) in EtOH (10 ml) was stirred at reflux temperature for 20 hr. The reaction mixture was filtered while hot and the filtrate evaporated in vacuo; the residue was dissolved in 5 ml of hot MeOH and, after standing at 10°, crystalline material formed which was washed with cold Et_2O and dried (yield 196 mg (70%), mp 176°). The product showed one spot on TLC in MeOH–$CHCl_3$ (1:9); R_f 0.66; ir λ_{max}^{KBr} 6.51 (1536) and 7.40 μ (1351 cm^{-1}) (CNO_2).

This method was also used to prepare **6** and **10** (Table VI).

Method C. O^6-Benzylinosine (14). Cleaned sodium metal (116 mg, 5.0 mmol) dissolved in dry benzyl alcohol (5 ml) was added to **73** (420 mg, 1.4 mmol) and heated at 85° with stirring for 1 hr. The reaction mixture was evaporated to dryness in vacuo at 60–70° and the residue dissolved in 5 ml of H_2O; this solution was adjusted to pH 6 with AcOH and extracted several times with EtOAc. The combined EtOAc extracts were evaporated to a small volume and treated with charcoal; upon the addition of petroleum ether, crystalline material formed at room temperature and was washed with Et_2O, dried, and recrystallized from EtOAc–petroleum ether [yield 480 mg (91.5%), mp 173°]. The product showed one spot on TLC in MeOH–$CHCl_3$ (1:9); R_f 0.60; ir λ_{max}^{KBr} 6.19 (1616) and 7.35 μ (1361 cm^{-1}).

Physical properties and chromatographic data for these and related compounds synthesized by the above methods are given in Tables VI–VIII.

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Synthesis and Cell Culture Studies on the Antiviral Activity of 5-Mercaptomethyl-2'-deoxyuridine†

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Treatment of 5-mercaptomethyluracil (I) with trimethylsilyl chloride in the presence of triethylamine gave 2,4,5-tris(trimethylsilyl)-5-mercaptomethyluracil (II) which, upon coupling with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranosyl chloride, furnished an anomeric mixture of fully substituted 2'-deoxy ribonucleosides. The nucleoside with β configuration (III) was predominantly formed and was isolated as a crystalline solid. The free nucleoside IV was obtained by removal of blocking groups by sodium methoxide catalyzed deacylation, deionization under reducing atmosphere, and chromatography on neutral alumina. IV is oxidized to the corresponding disulfide V in solution in the absence of thiols. IV was found to be markedly inhibitory against the herpes virus of infectious bovine rhinotracheitis (IBR). Against this virus, IV was found to be as potent as 5-iododeoxyuridine and cytosine arabinoside when added 18 hr before virus infection.

5-Hydroxymethylpyrimidines are unique components of the DNA of a virulent group of *Bacillus subtilis*[1-3] and *Escherichia coli* T-even bacteriophages.[4] 5-Substituted pyrimidines have also been postulated as intermediates during the conversion of thymine to RNA pyrimidines.[5] Antimetabolites of 5-hydroxymethyl-2'-deoxyuridine (OH-CH_2UdR) may show selective toxicity toward viruses if

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