Synthesis and Biological Evaluation of 2-Fluoro-8-azaadenosine and Related Compounds[1]

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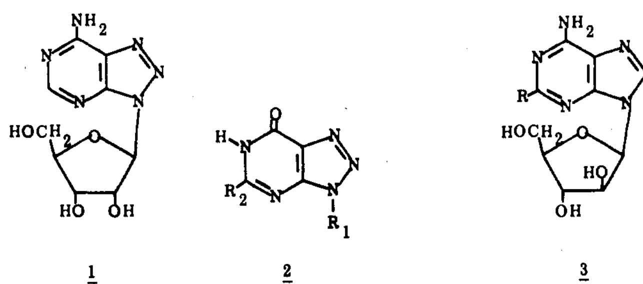
The synthesis of 2-fluoro-8-azaadenosine (**6e**) and 2-amino-8-azaadenosine (**6d**) is described. Condensation of 9*H*-2,6-bis(methylthio)-8-azapurine (**4**) with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (**5**) produces a mixture of **6a** (9-β-D-ribofuranosyl) and **7a** (8-β-D-ribofuranosyl). Standard functional group manipulation, including a modified Schiemann reaction to introduce the fluorine, allows preparation of **6d** and **6e** from the major isomer **6a**. By a similar series of reactions the minor component **7a** was converted to **7d** and **7e**, with the ribose ring attached at N-8 of the 8-azapurine ring system. Structure proofs utilized UV and ^{1}H and ^{13}C NMR data. Compounds **6b–e,g** and **7b–f** were evaluated in the H.Ep.-2 cell culture screen, and compounds **6c–e** and **7d** were evaluated in the P388 mouse leukemia screen. Adenosine deaminase data are also presented for some compounds.

The anticancer activity of 8-azapurine derivatives has been of continuing interest. The observance of in vivo activity for 8-azaadenosine (**1**, against L1210 and P388),[2,3]



a) R_1 = β-D-ribofuranosyl, R_2 = H
b) R_1 = H, R_2 = NH_2
c) R_1 = R_2 = H
d) R_1 = β-D-ribofuranosyl, R_2 = NH_2

a) R = F
b) R = H

8-azainosine (**2a**, against L1210 and adenocarcinoma 755),[2,4] and 8-azaguanine (**2b**, against L1210, adenocarcinoma 755, and others)[5-13] has served as the basis for the synthesis of a variety of congeners.[2,3,14-17] All three of these compounds are thought to be activated by conversion to the nucleotides of 8-azaadenosine or 8-azaguanosine, followed by incorporation into RNA. Metabolic studies in vitro have uncovered the pathways followed by **1**, **2a**, and **2b**.[4,18,19] 8-Azaadenosine is metabolized to the triphosphate and then incorporated into RNA. It is also rapidly deaminated to **2a** by adenosine deaminase and eventually also incorporated by way of 8-azaguanosine triphosphate.[19] In fact, **1a** has a much higher V_{max} than adenosine with the deaminase from calf intestine.[20] Activation of **2a** occurs by conversion to 8-azainosinic acid, either by direct phosphorylation by adenosine kinase or by cleavage to 8-azahypoxanthine (**2c**), which is then converted to the nucleotide by hypoxanthine phosphoribosyltransferase (HPRT). 8-Azainosinic acid is further converted to both 8-azaadenine and 8-azaguanine nucleotides.[4,19] 8-Azaguanine (**2b**) is converted by HPRT to the nucleotide directly.[19] After incorporation into RNA, it has been found to disrupt the protein synthesis machinery.[21-23] Marginal activity is seen in L1210 with 8-azaguanosine (**2d**), which is presumably metabolized through initial cleavage to 8-azaguanine by purine nucleoside phosphorylase, although limited direct phosphorylation may occur.[6]

Recent results have demonstrated an enhancement in cytotoxicity of 8-azaadenosine in several cell lines when these lines are pretreated with the adenosine deaminase inhibitor 2′-deoxycoformycin (pentostatin).[24-27] Other

(1) Throughout the text we have utilized the 8-azapurine designation to more readily relate the compounds discussed to the corresponding purines. The Experimental Section has the correct names for the compounds synthesized. Proper names for other compounds mentioned in the text are as follows: 2,6-bis(methylthio)-8-aza-9*H*-purine, 5,7-bis(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine; 8-azaadenosine, 3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine; 8-azainosine, 3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one; 8-azaguanine, 5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one; 8-azaguanosine, 5-amino-3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one.

(2) Montgomery, J. A.; Elliott, R. D.; Thomas, H. J. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 292.
(3) Elliott, R. D.; Montgomery, J. A. *J. Med. Chem.* **1977**, *20*, 116.
(4) Bennett, L. L., Jr.; Vail, M. H.; Allan, P. W.; Laster, W. R., Jr. *Cancer Res.* **1973**, *33*, 465.
(5) Kidder, G. W.; Dewey, V. C.; Parks, R. E.; Woodside, G. L. *Science* **1949**, *109*, 511.
(6) Montgomery, J. A.; Schabel, F. M., Jr.; Skipper, H. E. *Cancer Res.* **1962**, *22*, 504.
(7) Finkelstein, M.; Thomas, P. A. *Cancer Res.* **1951**, *11*, 801.
(8) Kidder, G. W.; Dewey, V. C.; Parks, R. E., Jr.; Woodside, G. L. *Cancer Res.* **1951**, *11*, 204.
(9) Gellhorn, A.; Engelman, M.; Shapiro, D.; Graff, S.; Gillespie, H. *Cancer Res.* **1950**, *10*, 170.
(10) Sigiura, K.; Hitchings, G. H.; Cavalieri, L. F.; Stock, C. C. *Cancer Res.* **1950**, *10*, 178.
(11) Law, L. W. *Cancer Res.* **1950**, *10*, 186.
(12) Shapiro, D. M.; Weiss, R.; Gellhorn, A. *Cancer* **1950**, *3*, 896.
(13) Goldin, A.; Greenspan, E. M.; Schoenback, E. B. *J. Natl. Cancer Inst.* **1950**, *10*, 319.
(14) Elliott, R. D.; Montgomery, J. A. *J. Med. Chem.* **1976**, *19*, 1186.
(15) Montgomery, J. A.; Thomas, H. J. *J. Org. Chem.* **1971**, *36*, 1962.
(16) Hutzenlaub, W.; Tolman, R. L.; Robins, R. K. *J. Med. Chem.* **1972**, *15*, 879.
(17) Davoll, J. *J. Chem. Soc.* **1958**, 1593.
(18) Smith, J. D.; Matthews, R. E. F. *Biochem. J.* **1957**, *66*, 323.
(19) Bennett, L. L., Jr.; Allan, P. W. *Cancer Res.* **1976**, *36*, 3917.
(20) Bennett, L. L., Jr.; Allan, P. W.; Hill, D. L.; Thomas, H. J.; Carpenter, J. W. *Mol. Pharmacol.* **1976**, *12*, 242.
(21) Rivest, R. S.; Irwin, D.; Mandell, H. G. *Biochem. Pharmacol.* **1982**, *31*, 2505.
(22) Grunberger, D.; Grunberger, G. *Antibiotics* **1979**, *5*, 110.
(23) Parks, R. E., Jr.; Agarwal, K. C. *Handb. Exp. Pharmacol.* **1975**, *28*, 450.
(24) Adamson, R. H.; Zaharevitz, D. W.; Johns, D. G. *Pharmacology* **1977**, *15*, 849.
(25) Chu, M. Y.; Dexter, D. L.; Melvin, J. B.; Robison, B. S.; Parks, R. E., Jr.; Calabresi, P. *Proc. Am. Assoc. Cancer Res.* **1980**, *21*, 269 (abstr 1080).
(26) Crabtree, G. W.; Dexter, D. L.; Spremulli, E. N.; Quevedo, W. C., Jr.; Calabresi, P.; Parks, R. E., Jr. *Proc. Am. Assoc. Cancer Res.* **1981**, *22*, 250 (abstr 990).

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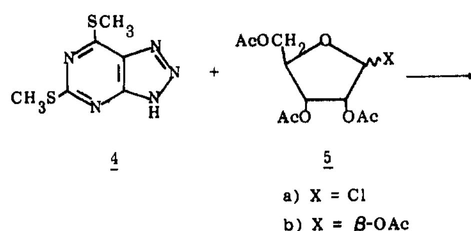
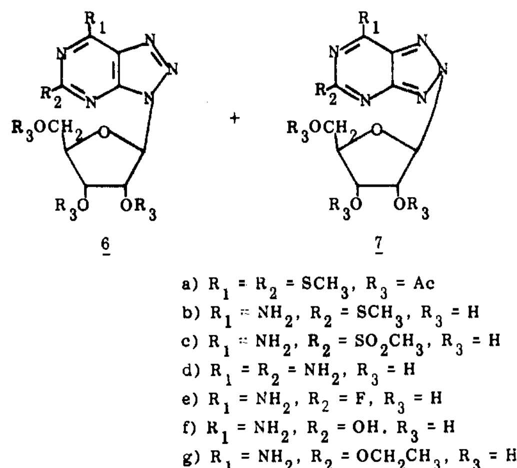
adenosine analogues that are normally deaminated have also shown a similar enhancement.[26,28] Rather than use pentostatin, which unfortunately has other effects also,[29,30] it is possible to greatly reduce deamination of an adenosine derivative by incorporating a 2-halo substituent.[31] The activity of 9-β-D-arabinofuranosyl-2-fluoroadenine (**3a**) in vivo against the murine leukemias L1210 and P388[32] is comparable to that of 9-β-D-arabinofuranosyladenine (**3b**) plus pentostatin.[33]

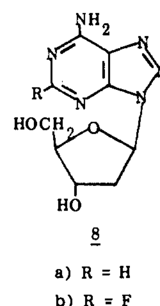
In light of the above cited observations, we were interested in determining the anticancer activity of 2-fluoro-8-azaadenosine (**6e**), an analogue of 8-azaadenosine (**1a**) which should undergo deamination significantly more slowly. This reduction in catabolism might result in an enhancement in cytotoxicity similar to that seen with 8-azaadenosine plus pentostatin. The chemical precursor of **1b**, 2-amino-8-azaadenosine (**6d**), was also of interest, since one would expect it to be deaminated to 8-azaguanosine. This paper describes the synthesis and biological evaluation of **6d**, **6e**, and some of the chemical intermediates leading to them.

Chemistry. Construction of a nucleoside suitable for the desired manipulations was accomplished by the molecular sieve catalyzed coupling of 2,6-bis(methylthio)-8-aza-9*H*-purine (**4**)[34] with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (**5a**) in toluene. This method was chosen since it is known to give β anomers in excellent yield in a similar case.[3] The crude mixture of nucleosides (**6a** and **7a**) was treated with ethanolic ammonia at room temperature for several days to effect displacement of the 6-methylthio group as well as O-deacetylation, producing the 9-substituted isomer **6b** and the 8-substituted isomer **7b**. Although no HPLC conditions were found to separate **6a**/**7a**, ratios of products could be determined by HPLC at the **6b**/**7b** stage. Separation was possible but extremely difficult at this stage. The **6**/**7** ratio was typically 6–8 to 1. At lower reaction temperatures, contrary to earlier observations with other 8-azapurines,[3] the ratio increased slightly to 7–9 to 1.

In order to facilitate incorporation of an amino group at C-2, the methylthio substituent was oxidized with *m*-chloroperoxybenzoic acid to a methylsulfonyl (**6c**/**7c**). At this stage the 9-isomer **6c** crystallized from the reaction mixture, enabling us to prepare the compounds in this series without a chromatographic separation of the isomers. Displacement of the methylsulfonyl occurred readily upon treatment with ethanolic ammonia at room temperature for 24 h, producing **6d**. Because of the difficulty in selective O-acylation of **6d**, we chose to carry out fluorine incorporation at C-2 directly by a modified Schiemann reaction,[35] rather than use the pyridine–hydrogen fluoride procedure,[36,37] which required blocking the carbohydrate







hydroxyls. Treatment of **6d** in 48% aqueous fluoboric acid with potassium nitrite produced the fluoro compound **6e** in moderate yield.

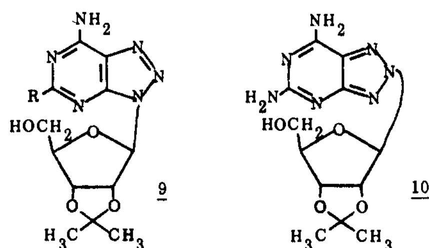
Evaluation of a sample of **7b** that was separated chromatographically from **6b** uncovered some cytotoxicity (vide infra); so we decided to prepare the 8-substituted 8-azapurine nucleosides **7d** and **7e**, also. Since the molecular sieve coupling gave only a small amount of **7a**, we searched for a coupling method that would result in a higher yield of **7a**. Fusion of **4** with 1,2,3,5-tetra-*O*-acetylribofuranose (**5b**) in the presence of a trace of *p*-toluenesulfonic acid afforded a mixture of nucleosides with a ca. 3:2 ratio of **6a**/**7a**. In addition, small amounts of other nucleosides, most likely the corresponding 8- and 9-substituted α anomers, were present. One of these minor components was isolated, converted to the isopropylidene derivative **9d**, and confirmed as an α isomer (see below). These minor components were removed during the purification procedures in subsequent steps. The crude reaction mixture from the fusion was treated with ethanolic ammonia, followed by *m*-chloroperoxybenzoic acid to afford **6c**/**7c**. Crystallization of the 9-isomer **6c** left a filtrate greatly enriched in the 8-isomer, which was carried on to the diamino compound **7d** with ethanolic ammonia. Purification by formation of the picrate allowed separation of the 8-isomer from the last traces of the 9-isomer (and any α

(27) Spremulli, E. N.; Crabtree, G. W.; Dexter, D. L.; Chu, S. H.; Farineau, D. M.; Ghoda, L. Y.; McGowan, D. L.; Diamond, I.; Parks, R. E., Jr.; Calabresi, P. *Biochem. Pharmacol.* **1982**, *31*, 2415.
(28) Johns, D. G.; Adamson, R. H. *Biochem. Pharmacol.* **1976**, *25*, 1441.
(29) Glazer, R. I. *Cancer Chemother. Pharmacol.* **1980**, *4*, 227.
(30) Plunkett, W.; Benjamin, R. S.; Keating, M. J.; Freireich, E. J. *Cancer Res.* **1982**, *42*, 2092.
(31) Montgomery, J. A. *Med. Res. Rev.* **1982**, *2*, 271, and references therein.
(32) Brockman, R. W.; Schabel, F. M., Jr.; Montgomery, J. A. *Biochem. Pharmacol.* **1977**, *26*, 2193.
(33) Brockman, R. W.; Cheng, Y.-C.; Schabel, F. M., Jr.; Montgomery, J. A. *Cancer Res.* **1980**, *40*, 3610.
(34) Montgomery, J. A.; Shortnacy, A. T.; Arnett, G.; Shannon, W. M. *J. Med. Chem.* **1977**, *20*, 401.
(35) Montgomery, J. A.; Hewson, K. *J. Org. Chem.* **1968**, *33*, 432.
(36) Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1982**, *59*, 2608.
(37) Olah, G. A.; Welch, J. T.; Vankar, Y. D.; Nojima, M.; Kerekes, I.; Olah, J. A. *J. Org. Chem.* **1979**, *44*, 3872.

isomers). Stirring the picrate with an anion exchange resin liberated **7d**. Formation of **7e** was conducted in the same manner as for **6e**. Purification of both fluoro compounds required the use of Bio-Beads SM-4 ion-exchange resin to separate inorganic material from the nucleoside. In the 8-substituted case, a considerable amount of the 2-hydroxy derivative **7f**, a byproduct in the reaction, was also isolated from the column.

NMR data for the various 8-azapurine derivatives are collected in Tables III–V. Proof that compounds in the **6** and **7** series are substituted at the 9- and 8-positions of the 8-azapurine ring, respectively, was obtained mainly from the ^{13}C NMR spectra. In the **6** series, compound **6c** showed a three-bond coupling between C_4 and H-1′ of 2.5 Hz in the coupled spectrum and no coupling between H-1′ and any other purine carbons. In the **7** series, compound **7c** showed no coupling between H-1′ and any of the 8-azapurine carbons. Also, as seen in the Experimental Section, the ultraviolet absorption spectra of the 8-substituted isomers, in particular, are quite characteristic.

In order to confirm that the major isomers were of the β configuration, **6d** and **7d** were both converted into the corresponding 2′,3′-*O*-isopropylidene derivatives (**9b** and



10, respectively),[38] and ^{1}H NMR and ^{13}C NMR spectra were recorded. For comparison purposes, 8-azaadenosine was similarly converted to its 2′,3′-*O*-isopropylidene derivative **9a**,[38] and spectra were recorded. A minor isomer (believed to be α) corresponding to **6b** was isolated in a mixture with **6b**, and the mixture of nucleosides was converted to the mixture of isopropylidene derivatives (**9c** and **9d**), which were separated chromatographically.[38] The data on all these compounds are reported in Tables III–V. A standard criterion for distinguishing α and β isomers is the occurrence of the chemical shift of H-1′ of an α isomer downfield from that of the corresponding β isomer.[39–42] Compounds **9c** and **9d** fit that pattern. Additionally, in agreement with another established criterion,[43] splitting of all four β isomers is >0.15 ppm, while that of α isomer **9d** is only 0.04 ppm. In the ^{13}C NMR, the 8-azapurine nucleosides (as their isopropylidene derivatives) do not fit the patterns seen previously for distinguishing α and β isomers.[44–46] Clear patterns are seen, however. The chemical shifts for the two isopropylidene methyls in the β isomers are very similar, as are the $\Delta\delta$ values (varying between 1.72 and 1.76 ppm), while for the α isomer **9d** the $\Delta\delta$ is only 0.48 ppm. Chemical shifts for the quaternary carbon of the isopropylidene group in the β isomers are in the 112.5–113.0-ppm range, while the shift for **9d** is 114.17 ppm. It appears that the lack of a substituent at the 8-position of the 8-azapurine ring is influencing the ribose ring conformation. Clear differences are also seen in the chemical shifts of C-1′. The chemical shift of C-1′ of **10** is in line with other 8-substituted compounds, while the three other β compounds are very similar but 2–3-ppm downfield for C-1′ of **9d**. That **9d** was substituted at the 9-position of the 8-azapurine was demonstrated by the three-bond coupling between H-1′ and C-4 ($J \simeq 1$ Hz) in the ^{13}C NMR spectrum, together with the lack of coupling between H-1′ and any other azapurine carbon.

Inspection of the data on the unblocked nucleosides **6a–e,g** and **7a–f** reveals several trends. In the ^{1}H NMR spectra of the 8-azaadenosine derivatives, the chemical shift of H-1′ for the 8-substituted compounds is upfield from the corresponding 9-substituted compounds, and $J_{1',2'}$ is consistently 1–1.5 Hz smaller in the **7** series than in the **6** series. Also, the chemical shift for $H_{2'}$ is upfield about 0.2 ppm in the **7** series relative to the **6** series, and all the values are quite similar within each series. In the ^{13}C NMR spectra, 9-substituted compounds are quite readily distinguishable from 8-substituted compounds by noting the position of C-1′. For the **7** series, C-1′ occurs at 95-98 ppm,

Table I. Cytotoxicity (H.Ep.-2 Cells) and P388 Screening Data

no.	IC_{50},[a] μM	added pentostatin IC_{50},[b] μM	P388[c] dose,[d] mg/kg	schedule	% ILS
1	0.7	<0.02			
2b	2				
2d	2				
6b	>130[e]				
6c	14		50 (400)	*f*	38
6d	0.7	>20[e]	6 (50)	*g*	25
6e	2	2	50 (200)	*f*	21
6g	>75[e]				
6h	0.8				
7b	25				
7c	22				
7d	>75[e]		400 (400)	*h*	0
7e	>75[e]				
7f	>75[e]				
8a	10	>25[e]			
8b	~70				

[a] The concentration required to inhibit the growth of cells to 50% of controls. See ref 47 for specific details. [b] 0.1 μg/mL of pentostatin added to the medium. [c] P388 leukemia cells (10^6) were implanted intraperitoneally (ip) in mice on day 0. Drug treatment began on day 1 by ip inoculation. The standard protocol of the Drug Evaluation Branch of the Division of Cancer Treatment, NCI, was followed (ref 48). [d] The dose listed is the optimum dose for this compound. The number in parentheses is the highest dose examined. [e] Highest drug concentration examined, with no toxic effects seen. [f] ip; qd 1-5. [g] ip; qd 1-9. [h] ip; day 1 only.

(38) The nucleosides 1 and 6b and the α isomer of 6b,d and 7d were converted to their 2′, 3′-*O*-isopropylidene derivatives under standard conditions (acetone, perchloric acid) and purified to homogeneity by recrystallization or preparative thick-layer chromatography. NMR spectra were recorded directly on these purified samples.

(39) Nishimura, T.; Shimizu, B. *Chem. Pharm. Bull.* **1965**, *13*, 803.
(40) Montgomery, J. A.; Thomas, H. J. *J. Am. Chem. Soc.* **1965**, *87*, 5442.
(41) Imai, K.; Nohara, A.; Honjo, M. *Chem. Pharm. Bull.* **1966**, *14*, 1377.
(42) Montgomery, J. A.; Hewson, K. *J. Med. Chem.* **1968**, *11*, 48.
(43) Tapiero, C.; Imbach, J.-L. *Nucleic Acid Chem.* **1978**, *2*, 1055.
(44) Ohrui, H.; Jones, G. H.; Moffatt, J. A.; Maddox, M. L.; Christensen, A. T.; Bryan, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 4602.
(45) Cousineau, T. J.; Secrist III, J. A. *J. Org. Chem.* **1979**, *44*, 4351.
(46) Tam, S. Y.-K.; Klein, R. S.; de las Heras, F. G.; Fox, J. J. *J. Org. Chem.* **1979**, *44*, 4854.

Table II. Adenosine Deaminase Kinetic Parameters[a]

substrate	K_m, μM	V_{max}, μmol mg^{-1} min^{-1}
adenosine[b]	29	435
8-azaadenosine[b] (1)	250	1500
8-aza-2′-deoxyadenosine (8a)[c]	220	2000
ara-A[b]	120	94
8-aza-*ara*-A[b]	430	75
6-methoxypurine riboside[d]	50	2
8-aza-6-methoxypurine riboside[d]	140	27
2-aminoadenosine[c]	33	119
2-amino-8-azaadenosine[c] (6d)	12	23
O^6-methylguanosine[e]	42	61
8-aza-O^6-methylguanosine[d]	22	20
2-fluoroadenosine[c]	81	0.78
2-fluoro-8-azaadenosine[c] (6e)	250	5

[a] Data for all compounds refer to calf intestine adenosine deaminase. See ref 20 for experimental details. [b] See ref 20. [c] L. L. Bennett, Jr., and P. W. Allan, unpublished results. Assays were carried out by standard procedures, as reported in ref 20. [d] See ref 31. [e] See ref 52.

while in the **6** series it occurs at 87–90 ppm. The difference between the two corresponding isomers is 8.0 ± 0.5 Hz. Also, in the carbohydrate ring the chemical shifts for C-2′ are 1–2 ppm lower field for the **7** series (ca. 73.8–74.8 ppm) than for the **6** series (72.5–73 ppm). In the 8-azapurine ring, the chemical shifts of C-4, C-5, and C-6 of the 9-substituted 8-azaadenine derivatives are consistently upfield from the corresponding 8-substituted 8-azaadenine derivatives, with C-4 showing the largest shift (6.8–8.5 ppm). The presence of fluorine at C-2 in **6e** and **7e** causes the splitting of all of the ring carbon signals, and Table V provides the coupling constants, which are consistent with the number of bonds involved.

Biological Evaluation. Compounds in both the 8- and 9-ribofuranosyl series were initially evaluated for cytotoxicity in the H.Ep.-2 cell culture screen,[47] and the results are shown in Table I. Compounds **6c–e**, **7b,c**, and **8a,b** had cytotoxicity at the concentrations employed, though only our initial target structures **6d** and **6e** exhibited marked cytotoxicity.

Of the 8-substituted compounds, only the two sulfur-containing nucleosides **7b** and **7c** were toxic, while the 2-amino and 2-fluoro compounds in this series were not toxic at the levels examined. Only **6b** of all the sulfur-containing nucleosides did not exhibit some cytotoxicity. Compounds **6b** and **7b–d** are not substrates for adenosine deaminase, while **6c** is an extremely poor substrate.

The diamino compound **6d** is about as cytotoxic as 8-azaadenosine (**1a**) and slightly more toxic than 8-azaguanosine (**2d**). Since both 8-azaadenosine and 2-aminoadenosine are excellent substrates for adenosine deaminase (see Table II), it seemed likely that **6d** was being converted rapidly to **2d**, and **6d** proved to be an excellent substrate for the enzyme (Table II). Thus, the cytotoxicity of **6d** presumably results from rapid deamination to 8-azaguanosine, which is then metabolized via 8-azaguanine to the nucleotide level. In agreement with this suggestion, **6d** is not cytotoxic at the highest levels examined when incubated with pentostatin.

As seen in Table I, incubation of 8-azaadenosine with pentostatin in H.Ep.-2 cells does considerably increase the cytotoxicity. Compound **6e** is somewhat less cytotoxic than 8-azaadenosine, and incubation with pentostatin results in no change in the IC_{50}. Thus, it appears that the cytotoxicity of **6e** is probably caused by conversion to the corresponding nucleotide. Data with adenosine deaminase (Table II) shows **6e** to be a relatively poor substrate. Experimentally, **6e** is deaminated completely over a few hours under conditions where adenosine is deaminated in several minutes.

The 2′-deoxyribofuranoside **8b**, prepared from 2-fluoro-8-azaadenine,[49] was markedly less cytotoxic than either the ribofuranoside **6e** or 8-aza-2′-deoxyadenosine (**8a**).[51] Compound **8a** presumably causes most of its toxic effects by initial deamination (see Table II), since incubation with pentostatin produces an increased IC_{50} (no toxicity was seen at the highest levels examined). It is worthy of note that with the 8-azaadenine derivatives examined, the 2′-deoxy compounds are less cytotoxic than the corresponding ribose derivatives.

Compounds **6c–e** and **7d** were examined against the P388 leukemia in mice, with only modest increases in life span seen for **6c** and **6d**. The activity of **6d**, which is probably deaminated to 8-azaguanosine in vivo, is similar to that seen with 8-azaguanosine against the L1210 leukemia in mice.[6] It is interesting to note that the methylsulfonyl compound **6c** showed the greatest increase in life span of all of the compounds examined.

Kinetic data for the deamination of various 8-azapurine derivatives is contrasted to the data for the corresponding purine derivatives in Table II. In examples where the 2-position is unsubstituted, or substituted with a fluorine, the binding of the substrates to the enzyme is considerably poorer with the 8-azapurine derivatives than with the purine derivatives, as reflected in the K_m values, while the V_{max} values for the 8-azapurines range from comparable to considerably higher than for the purines. When a 2-amino group is present, however, the binding of the substrates in the 8-azapurine derivatives is tighter than with the purine derivatives, while the V_{max} values, on the other hand, are considerably lower. A comparison of 8-azaadenosine (**1**) and the 2-fluoro derivative **6e** shows them to have the same value for K_m, while the V_{max} for **1** is 300-fold higher than that of **6e**.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded in dimethyl-d_6 sulfoxide with a Varian XL-100-15 spectrometer operating at 100.1 MHz for ^1H and 25.16 MHz for ^{13}C. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Chemical shifts are tabulated in Tables III and IV and coupling constants in Table V.

Ultraviolet absorption spectra were determined with a Cary 17 spectrophotometer. Each compound was dissolved in ethanol and diluted tenfold with 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH. Numbers in parentheses are extinction coefficients ($\epsilon \times 10^{-3}$). Microanalyses were performed by Galbraith Laboratories,

(47) Bennett, L. L., Jr.; Vail, M. H.; Allan, P. W.; Shaddix, S. C. *Biochem. Pharmacol.* **1973**, *22*, 1221.
(48) Protocol 1.200, *Cancer Chemotherap. Rep., Part 3* **1972**, *3*, 9.
(49) The preparation of **8b** was accomplished in collaboration with Drs. R. L. Blakley and M.-C. Huang. 2-Fluoro-8-azaadenine was prepared in 10–20% yield by treatment of 2,6-diamino-8-azapurine in hydrogen fluoride–pyridine (70:30) at 10 °C with sodium nitrite (3 equiv). Conversion to the 2′-deoxynucleoside was accomplished by incubation with nucleoside deoxyribosyltransferase from *L. leishmanii* with thymidine as the 2′-deoxyribose donor. Experimental procedures and purifications were as described for other compounds in ref 50.
(50) Huang, M.-C.; Hatfield, K.; Roetker, A. W.; Montgomery, J. A.; Blakley, R. L. *Biochem. Pharmacol.* **1981**, *30*, 2663.
(51) Montgomery, J. A.; Thomas, H. J. *J. Med. Chem.* **1972**, *15*, 305.
(52) Pegg, A. E.; Swann, P. F. *Biochim. Biophys. Acta* **1979**, *565*, 241.

Table III. 100-MHz ^{1}H NMR Chemical Shifts[a]

no.	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$, $H_{5'}$	2'-OH	3'-OH	5'-OH	other
6a	6.59 (d)	6.13 (dd)	5.82 (s)	4.02–4.62 (m)					1.93, 2.12, 2.14 (3s, $COCH_3$), 2.67, 2.77 (2 s, 2 SCH_3)
6b	6.11 (d)	*b*	4.33 (m)	4.00 (m)	3.35–3.80 (m)	5.55 (d)	5.27 (d)	4.74–5.00 (m)	2.51 (s, SCH_3), 8.24, 8.46 (2 br s, NH_2)
6c	6.21 (d)	*b*	4.37 (m)	4.05 (m)	3.4–3.8 (m)	5.61 (d)	5.33 (d)	4.75–5.04 (m)	3.38 (s, SO_2CH_3), 8.99, 9.22 (2 br s, NH_2)
6d	5.96 (d)	4.80 (m)	4.26 (m)	3.97 (m)	3.35–3.78 (m)	5.16 (br s)	5.47 (br s)	5 (br, hidden)	6.43, 7.65 (2 br s, 2 NH_2)
6e	6.07 (d)	*b*	4.32 (m)	4.03 (m)	3.32–3.80 (m)	5.59 (d)	5.29 (d)	4.80–4.96 (m)	8.70, 9.05 (2 br s, NH_2)
6g	6.06 (d)	*b*	4.25 (m)	3.99 (m)	3.4–3.8 (m)	5.52 (d)	5.25 (d)	4.75–4.98 (m)	4.34 (q, OCH_2), 1.32 (t, CH_3), 8.08, 8.38 (2 br s, NH_2)
7a	6.64 (d)	*c*	*c*	*c*	*c*				2.60 (s, SCH_3), $COCH_3$(c), 2.72 (s, SCH_3)
7b	6.03 (d)	4.59 (m)	4.34 (m)	4.05 (m)	3.40–3.78 (m)	5.67 (d)	5.27 (d)	4.77 (t)	2.47 (s, SCH_3), 8.28 (br s, NH_2)
7c	6.15 (d)	4.62 (m)	4.36 (m)	4.09 (m)	3.4–3.8 (m)	5.77 (d)	5.36 (d)	4.77 (t)	3.35 (s, SO_2CH_3), 9.05, 9.15 (2 br s, NH_2)
7d	5.91 (d)	4.55 (m)	4.29 (m)	4.01 (m)	3.26–3.80 (m)	5.59 (d)	5.21 (d)	4.76 (t)	6.17, 7.64 (2 br s, 2 NH_2)
7e	6.07 (d)	4.62 (m)	4.33 (m)	4.07 (m)	3.4–3.8 (m)		4.77, 5.31, 5.69 (3 br s)		8.84 (br s, NH_2)
7f	5.99 (d)	4.55 (m)	4.26 (m)	4.01 (m)	3.36–3.74 (m)		4.77, 5.38, 5.63 (3 br s)		~9 (v br s, NH_2, 2-OH)
9a	6.45 (d)	5.64 (dd)	5.09 (dd)	4.30 (m)	3.24–3.68 (m)			5.02 (t)	1.38, 1.57 [$C(CH_3)_2$], ~8.4 (NH_2), 8.39 (H-2)
9b	6.17 (d)	5.52 (dd)	5.05 (dd)	4.21 (m)	3.24–3.68 (m)			~5.0 (br)	1.36, 1.54 [$C(CH_3)_2$], 6.53, 7.67 (2 NH_2)
9c	6.38 (d)	5.63 (dd)	5.07 (dd)	4.25 (m)	3.24–3.66 (m)			4.91 (t)	1.37, 1.56 [$C(CH_3)_2$], 8.28, 8.47 (NH_2), 2.54 (SCH_3)
9d	6.70 (d)	5.12 (m)	4.93 (dd)	4.76 (m)	3.5–3.85 (m)			~5.1 (br)	1.19, 1.23 [$C(CH_3)_2$], 8.25 (NH_2), 2.53 (SCH_3)
10	6.23 (br s)	5.43 (dd)	5.00 (dd)	4.26 (m)	3.26–3.7 (m)			4.92 (t)	1.36, 1.53 [$C(CH_3)_2$], 6.23, 7.69 (2 NH_2)

[a] In Me_2SO-d_6. Chemical shifts are in parts per million downfield from internal tetramethylsilane. Decoupling was used to make the assignments. [b] The resonance for $H_{2'}$ is included in the multiplet for the 5'-OH. [c] Hidden under the corresponding signal for **6a**.

Knoxville, TN, or in the Molecular Spectroscopy Section of Southern Research Institute. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Thin-layer chromatography was carried out on Analtech precoated (250 μm) silica gel (GF) plates. Solvent systems listed in parentheses after the R_f value are as follows: A, 98:2 $CHCl_3$–CH_3OH; B, 5:1 $CHCl_3$–CH_3OH; C, 3:1 $CHCl_3$–CH_3OH; D, 4:1 $CHCl_3$–CH_3OH. In all cases, the term ethanol refers to absolute ethanol.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-5,7-bis(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (6a). A mixture of 2,6-bis(methylthio)-8-azapurine (20 g, 94 mmol)[34] and 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (42 g, 143 mmol) in 2 L of dry toluene containing 200 g of AW-500 molecular sieves (4Å molecular sieves were also successfully employed) was heated under reflux with stirring for 0.5 h. Another 200 g of sieves was added to the resulting solution, and heating was continued for a total reaction time of 18 h. The reaction mixture was treated with charcoal and filtered, and the filtrate was washed with saturated $NaHCO_3$ solution and H_2O and then dried over $MgSO_4$. The residue after evaporation (40 g) was used without further purification in the next step.

3-β-D-Ribofuranosyl-5-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (6b). A solution of **6a** (40 g, 84 mmol) in 1 L of ethanol saturated with NH_3 at 5 °C was allowed to stand in a bomb at room temperature for 4 days before it was evaporated to dryness. A second run was heated at 90 °C for 16 h with comparable results. The residue was recrystallized from 10% aqueous ethanol to afford an overall yield for the two steps of 10.6 g (40%). This material was used in the next step. A second recrystallization yielded an analytical sample, mp 204–205 °C. The mother liquors yielded 5 g of a 2- and 3-isomer mixture containing approximately 30% of the 2-isomer: R_f 0.67 (A); UV λ_{max} at pH 1, 241 (13.8), 277 nm (16.1); at pH 7, 247 (20.0), 288 nm (13.4); at pH 13, 247 (20.5), 288 nm (13.4). Anal. ($C_{10}H_{14}N_6O_4S\cdot1H_2O$) C, H, N.

3-β-D-Ribofuranosyl-5-(methylsulfonyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (6c). A solution of **6b** (8 g, 25.5 mmol) in 1.8 L of ethanol at 5 °C was treated dropwise over 1 h with *m*-chloroperoxybenzoic acid (22.8 g, 112 mmol) in ethanol (200 mL). The reaction was allowed to warm to room temperature and stir for 19 h before the precipitated white solid was collected, washed with ethanol, and dried to yield 7 g (80%). A second crop was obtained from the evaporated filtrate after ether trituration and ethanol recrystallization of the residue: yield 873 mg (9%). The analytical sample was obtained by recrystallization from ethanol: mp 218–220 °C dec; R_f 0.41 (B); UV λ_{max} at pH 1, 258 (sh), 263 (sh), 288 nm (9.8); at pH 7, 258 (sh), 263 (sh), 288 nm (9.9); at pH 13, 255 (sh), 290 nm (10.7). Anal. ($C_{10}H_{14}N_6O_6S$) C, H, N.

3-β-D-Ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-5,7-diamine (6d). A mixture of **6c** (2.0 g, 5.7 mmol) and ethanol (300 mL) previously saturated with NH_3 at 5 °C was allowed to stand at room temperature in a pressure bomb for 2 days (a second run heated at 55 °C for 16 h gave comparable results). A white solid was recovered after evaporation and ethanol trituration: yield 1.5 g (85%). The analytical sample was obtained from methanol: mp 193–195 °C; R_f 0.40 (C); UV λ_{max} at pH 1, 257 (11.5), 283 nm (8.1); at pH 7, 225.5 (22.2), 256 (sh), 285 nm (10.8); at pH 13, 225.5 (22.0), 257 (sh), 286 nm (10.6). Anal. ($C_9H_{13}N_7O_4\cdot0.5H_2O$) C, H, N.

Another nucleoside, **6g**, was isolated from the mother liquor by preparative thick-layer chromatography (Analtech, 1 mm, silica gel, elution three times with 5:1 $CHCl_3$-CH_3OH) and crystallization from acetonitrile–water: mp 207–209 °C; R_f 0.62 (B); UV λ_{max} at pH 1, 270 nm (9.9); at pH 7, 273 nm (7.6); at pH 13, 275 nm (7.7). Anal. ($C_{11}H_{16}N_6O_5$) C, H, N.

3-β-D-Ribofuranosyl-5-fluoro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (6e). Solid **6d** (622 mg, 2.2 mmol) was added to 30 mL of 48% HBF_4 at 5 °C and stirred until the solid dissolved. To the resulting solution was added solid KNO_2 (467 mg, 5.5 mmol) portionwise over 1 h. At 1 h after completion of the addition, HPLC indicated an absence of starting material. The reaction was chilled from 5 to −25 °C and then neutralized with 11.7 M KOH, allowing the temperature to increase gradually to 10 °C. The resulting mixture was chilled for 1 h, salts were collected, and the filtrate was diluted to 150 mL and passed

Table IV. 25.16-MHz ^{13}C NMR Chemical Shifts[a]

no.	$C_{1'}$	$C_{2'}$[b]	$C_{3'}$[b]	$C_{4'}$	$C_{5'}$	C_2	C_4	C_5	C_6	other
6a	87.46	72.55	70.02	79.73	62.32	170.74	147.61	131.81	164.71	11.60, 14.23 (2 SCH_3), 20.26 ($COCH_3$), 169.27, 169.45, 169.83 ($COCH_3$)
6b	89.37	72.58	70.67	85.85	61.91	170.42	149.70	122.65	154.97	13.63 (SCH_3)
6c	89.66	72.97	70.52	86.17	61.62	163.55	148.69	124.42	156.70	39.06 (SO_2CH_3)
6d	88.66	72.58	70.81	85.74	62.12	162.63	151.66	120.40	156.13	
6e	89.53	72.87	70.58	86.11	61.78	161.34	151.11	123.64	157.90	
7a	95.10	73.83	70.30	80.60	c	169.64	156.11	131.94	165.74	$COCH_3$(e), 11.77, 14.13 (2 SCH_3)
7b	97.36	74.72	70.68	86.35	61.99	169.94	158.23	124.50	155.41	13.50 (SCH_3)
7c	98.06	74.98	70.61	86.66	61.86	164.04	156.67	126.08	157.84	38.86 (SO_2CH_3)
7d	96.74	74.46	70.72	86.04	62.14	162.61	160.09	122.96	156.63	
7e	97.64	74.76	70.65	86.53	61.94	161.58	157.92	125.08	159.17	
7f	96.49	74.23	70.54	86.13	61.92	156.99[d]	156.50[d]	121.86 (br)	150.83[a]	
9a	90.56	81.85[b]	83.01[b]	88.05	61.27	157.13	148.84	123.98	156.30	25.08, 26.83 (2 CH_3), 112.97 [$C(CH_3)_2$]
9b	89.54	81.89[b]	82.88[b]	87.78	61.35	151.59[d]	156.19[d]	120.24	162.92[d]	25.11, 26.84 (2 CH_3), 112.75 [$C(CH_3)_2$]
9c	90.25	81.85[b]	82.96[b]	88.23	61.22	170.75	149.47	122.50	155.02	25.02, 26.78 (2 CH_3), 112.88 [$C(CH_3)_2$], 13.67 (SCH_3)
9d	87.83	80.16[b]	81.58[b]	84.65	62.07	169.83	149.78	121.98	154.92	24.48, 24.95 (2 CH_3), 114.17 [$C(CH_3)_2$], 13.63 (SCH_3)
10	97.15	81.91[b]	83.81[b]	88.63	61.37	156.66[d]	160.28[d]	123.26	162.80[d]	24.95, 26.67 (2 CH_3), 112.58 [$C(CH_3)_2$]

[a] In Me_2SO-d_6. Chemical shifts are in parts per million downfield from internal tetramethylsilane. Decoupling was used to make the assignments. [b] Assignments of $C_{2'}$ and $C_{3'}$ may be reversed. [c] Hidden under the corresponding signal for 6a. [d] In this compound, the assignments of these carbons may be revised.

Table V. First-Order Coupling Constants[a]

no.	$J_{1',2'}$, Hz	$J_{2',3'}$, Hz	$J_{2',OH}$, Hz	$J_{3',OH}$, Hz	$J_{5',OH}$, Hz
6a	3	5.5			
6b	5		5.5	5	
6c	5		5	5	
6d	5				
6e[b]	5		6	5.5	
6g	5		6	5	
7a	2				
7b	4		5.5	5.5	5.5
7c	3.5		5.5	6	5.5
7d	3.5		5.5	5.5	5
7e[c]	4				
7f	4				
9a	2	6			
9b	2	6			
9c	2	6			
9d	5.5	6.5			
10	1	6			

[a] In Me_2SO-d_6. [b] $^1J_{C_2F_2}$ = 209.6; $^3J_{C_4F_2}$ = 22.4; $^3J_{C_6F_2}$ = 23.2; $^4J_{C_5F_2}$ = 3.7 Hz. [c] $^1J_{C_2F_2}$ = 206.2; $^3J_{C_4F_2}$ = 21.9; $^3J_{C_6F_2}$ = 27.4; $^4J_{C_5F_2}$ = 3.4 Hz.

through a 15 × 25 cm bed of Bio-Beads, SM-4, 20–50 mesh, 25 g, prepared in H_2O. The adsorption at 1 mL/min was followed by a water wash until the eluate was free of potassium ions. The fluorine-containing nucleoside was obtained by methanol elution, followed by chromatography on Avicel F plates in CH_3CH_2OH–H_2O, 3:2, and recrystallization from H_2O to yield 135 mg (21%). The analytical sample was obtained by an additional H_2O recrystallization: mp 218–220 °C dec; R_f 0.64 (D); UV λ_{max} at pH 1, 270 nm (13.1); at pH 7, 270 nm (13.2); at pH 13, 271.5 nm (11.0). Anal. ($C_9H_{11}FN_6O_4$) C, H, N.

2-β-D-Ribofuranosyl-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-5,7-diamine (7d). Preparation of **7d** proved most expeditious by carrying the sequence straight through with only crude purification until the diamino stage. We have indicated, however, how we obtained pure **7b** and **7c** for analytical data accumulation and biological evaluation. Compound **7a** was never obtained free of **6a**.

A mixture of 4 (5 g, 23.4 mmol), **5b** (11.2 g, 35.2 mmol), and *p*-toluenesulfonic acid (286 mg, 1.5 mmol) was stirred mechanically at room temperature for 10 min at 25 mm before immersion in a preheated 120 °C oil bath. A clear melt was obtained after 5 min, with stirring and heating at 25 mm continued for an additional 10 min. The melt, after cooling, was dissolved in $CHCl_3$, washed with saturated aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl, dried over $MgSO_4$, and evaporated to dryness, leaving a dark residue containing **7a** and **6a**.

A solution of the dark residue in 400 mL of ethanol saturated with ammonia at 5 °C was heated in a pressure bomb at 90 °C for 16 h. Evaporation of the reaction mixture to dryness, followed by recrystallization from ethanol (charcoal), gave a solid that was approximately 1:1 **7b**/**6b**. The filtrate contained **6b**, **7b**, and the two corresponding α isomers in small amounts. The solid and the filtrate were both carried on to **7c** and **7d** separately. Pure **7b** was obtained free of **6b** by preparative TLC [Whatman, silica gel plates (1 mm) with a Celite spotting zone], using three successive plates, eluting with $CHCl_3$–CH_3OH (5:1), with two elutions on the last plate and followed by recrystallization of the plate band from methanol. **7b**: UV λ_{max} at pH 1, 230 (12.4), 245 (sh), 284 (13.4), 305 nm (12.7); at pH 7, 224 (15.1), 247 (13.5), 279 (9.8), 308 nm (9.5); at pH 13, 222 (14.4), 246 (15.5), 277 (10.4), 308 nm (10.4). Anal. ($C_{10}H_{14}N_6O_4{\cdot}0.5H_2O$) C, H, N.

The filtrate and the solid nucleoside mixture in ethanol were chilled separately to 5 °C and treated dropwise with *m*-chloroperoxybenzoic acid (4.4 equiv), in ethanol. After stirring at room temperature for 18 h, the mixtures were chilled, and the precipitated **6c** was removed by filtration. The filtrates were evaporated to dryness and then triturated with ether to remove excess peracid plus most of the *m*-chlorobenzoic acid. Pure **7c** was obtained by preparative TLC of the residue after trituration (Whatman silica gel plate, 1 mm), eluting four times with $CHCl_3$–CH_3OH (5:1), followed by recrystallization of the plate band from ethanol. **7c**: UV λ_{max} at pH 1, 227 (13.7), 255 (3.5), 301 nm (9.6); at pH 7, 226 (14.2), 255 (3.6), 300 nm (9.8); at pH 13, 315 (7.3), 334 nm (sh). Anal. ($C_{10}H_{14}N_6O_6S$) C, H, N.

The residues after trituration were dissolved separately in ethanol saturated with ammonia at 5 °C, and each was placed in a pressure bomb for 20 h at 55 °C. After evaporation of solvent, the residues were taken up in hot ethanol and treated with ~1 equiv of picric acid in ethanol. After concentrating and chilling, the precipitated picrates (mainly **7d** plus a trace of **6d**) were collected. The picrate of **7d** was obtained pure by recrystallization of the crude picrate mixtures from water. Liberation of **7d** was accomplished by stirring a methanol–water solution of the combined picrates with Dowex IX-8 (carbonate form) to remove picric acid, followed by filtering, evaporating, and recrystallizing the residue from methanol to afford 668 mg (13%) of **7d**, mp amorphous. In the case of the picrate mixture which contains the α isomers, recyrstallization did not remove the α isomer of

7d. After liberation of **7d** from the picrate, recrystallization from methanol initially yielded pure **7d**. Later crops contained some of the α isomer of **7d**, along with **7d**. **7d**: UV λ_{max} at pH 1, 264 (13.2), 286 nm (12.8); at pH 7, 261 (5.7), 314 nm (8.2); at pH 13, 261 (5.7), 313 nm (8.5). Anal. ($C_9H_{13}N_7O_4 \cdot 0.5H_2O$) C, H, N.

2-β-D-Ribofuranosyl-5-fluoro-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (7e). Solid **7d** (220 mg, 0.78 mmol) was added to 8 mL of 48% HBF_4 at 10 °C. The resulting stirred solution was treated with solid KNO_2 (1.3 g, 15.6 mmol) over 3.7 h at 10 °C, at which time HPLC showed only a trace of starting material. The reaction was chilled to −20 °C and neutralized to pH 7 with 11.7 M KOH. After chilling, the precipitated salts were collected, and the filtrate was passed at 1 mL/mm through a 1.5 × 24 cm bed of Bio-Beads, SM-4, 20–50 mesh, which had been prepared in H_2O. The column was eluted with H_2O until the eluate was free of potassium ions. The product was isolated from a H_2O–CH_3OH (3:1) eluate, followed by chromatography on an Avicel F plate in CH_3CH_2OH–H_2O (3:2). The methanol extract of the plate band was evaporated and triturated with cold CH_3CN to give a white solid (32 mg, 14%), mp 190 °C dec. **7e**: UV λ_{max} at pH 1, 247 (5.5), 292 nm (10.0); at pH 7, 247 (5.6), 292 nm (10.2); at pH 13, 243 (4.6), 297 nm (9.3). Anal. ($C_9H_{11}FN_6O_4 \cdot 0.25H_2O$) C, H, N.

Another nucleoside, **7f**, was isolated from an aqueous column fraction by evaporation and trituration of the residue with CH_3OH: yield 70 mg, mp amorphous.

7f: UV λ_{max} at pH 1, 291 nm (9.7); at pH 7, 262 (10.4), 287 nm (11.0); at pH 13, 259 (5.1), 317 nm (8.1). Anal. ($C_9H_{12}N_6O_5 \cdot 0.7H_2O$) C, H, N.

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Registry No. 4, 61772-86-9; **5a**, 40554-98-1; **5b**, 13035-61-5; **6a**, 86480-35-5; **6b**, 86480-36-6; **6c**, 86480-37-7; **6d**, 70421-27-1; **6e**, 86480-39-9; **6g**, 86480-38-8; **7a**, 86480-41-3; **7b**, 86480-42-4; **7c**, 86480-43-5; **7d**, 86480-40-2; **7d** picrate, 86480-44-6; **7e**, 86480-45-7; **7f**, 86480-46-8; **9a**, 53458-40-5; **9b**, 86480-47-9; **9c**, 86480-48-0; **9d**, 86480-49-1; **10**, 86480-50-4.

Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides as Anticoccidials. 3. Synthesis and Activity of Some Nucleosides of 4-[(Arylalkenyl)thio]pyrazolo[3,4-*d*]pyrimidines

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Ribonucleosides of 4-(alkylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidines have been shown to be useful anticoccidial agents [Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon, R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32. Rideout, J. L.; Krenitsky, T. A.; Elion, G. B. U.S. Patent 4 299 283, 1981]. In that study, the unsaturated 4-allylthio and 4-crotylthio derivatives (**19** and **20**) were shown to be more active in vivo against *Eimeria tenella* than their saturated congeners; therefore, some unsaturated (arylalkyl)thio derivatives were synthesized and investigated as anticoccidial agents. The novel compounds in this study (**2** to **18**) were prepared by the alkylation of 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**), which was prepared by an enzymatic method. The (*E*)-4-cinnamylthio derivative (**2**) and the 5′-monophosphate (**18**) were the most active compounds against *E. tenella* in vivo. None of the analogues with substituents in the aryl moiety (**3** to **13**) was more active than **2** in vivo. The geometry about the double bond was important, since the (*Z*)-4-cinnamylthio derivative (**14**) was inactive both in vitro and in vivo. The 4-(3-phenylpropynyl)thio and 4-(5-phenyl-2,4-pentadienyl)thio derivatives (**15** and **16**) were at least as active as **2** in vitro; however, they were less active than **2** in vivo. Compound **2** was effective in vivo against *E. tenella*, *E. necatrix*, *E. maxima*, and *E. brunetti*; these species of *Eimeria* were controlled when **2** was given in the diet at levels up to 100 ppm. Infections in vivo due to *E. acervulina* were controlled by **2** only at about 800 ppm. The broad spectrum of anticoccidial activity shown by **2** represents a significant improvement over the activities reported for related compounds [Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon, R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32].

Previous reports[1,2] have shown that ribonucleosides of 1*H*-pyrazolo[3,4-*d*]pyrimidines were able to inhibit the development of *Eimeria tenella* in chicks. It was shown that the alkylthio derivatives[1] were superior to the alkylamino derivatives.[2] In the study with the 4-alkylthio compounds,[1] it was apparent that unsaturation in the alkyl chain of the 4-substituent enhanced the activity in vivo relative to that found for the saturated congeners. The study has therefore been extended to include 4-(arylalkenyl)thio and 4-(arylalkynyl)thio analogues. The beneficial effects of these modifications and structure–activity relationships will be discussed.

Results and Discussion

Chemistry. The compounds (**2** to **16**, Table I) were prepared by the alkylation of 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine[3] (**1**) with the ap-

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(1) (a) Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32. (b) Rideout, J. L.; Krenitsky, T. A.; Elion, G. B. U.S. Patent 4 299 823 (1981).
(2) Rideout, J. L.; Krenitsky, T. A.; Koszalka, G. W.; Cohn, N. K.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 1040.

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