4',5'-Unsaturated 5'-Halogenated Nucleosides. Mechanism-Based and Competitive Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase

Esa T. Jarvi, James R. McCarthy,* Shujaath Mehdi, Donald P. Matthews, Michael L. Edwards, Nellikunja J. Prakash,* Terry L. Bowlin, Prasad S. Sunkara, and Philippe Bey

Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215. *Received May 29, 1990*

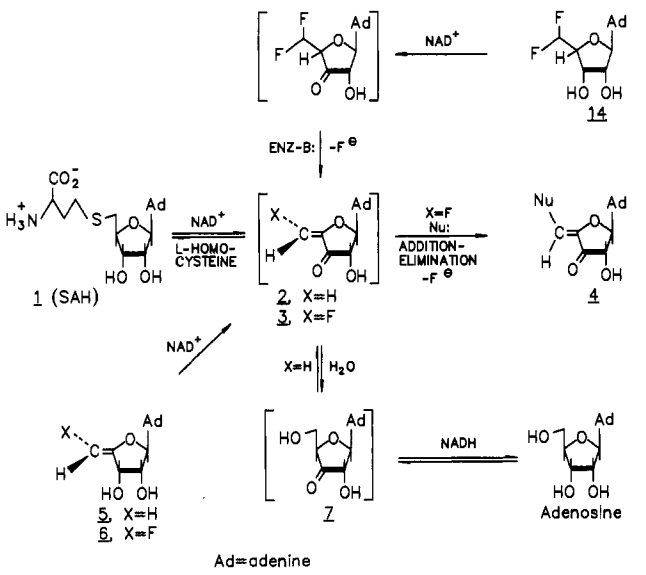
The design and synthesis of (*E*)- and (*Z*)-5'-fluoro-4',5'-didehydro-5'-deoxyadenosine (**6** and **13**, respectively), a new class of mechanism-based inhibitors of *S*-adenosyl-L-homocysteine (SAH) hydrolase, is described. A number of analogues of **6** and **13** were synthesized in order to determine the structure–activity relationship necessary for inhibition of the enzyme. Substitution of chlorine for fluorine in **6** (i.e. **44**), addition of an extra chlorine to the 5'-vinyl position (i.e. **51** and **52**), modification of the 2'-hydroxyl group to the deoxy (**34** and **35**) and arabino (**36** and **37**) nucleosides provided competitive inhibitors of SAH hydrolase. Nucleosides **6** and **13**, as well as 5'-deoxy-5',5'-difluoroadenosine (**14**) proved to be time-dependent inhibitors of SAH hydrolase. All three compounds are postulated to inhibit through the potent electrophile derived from oxidation of the 3'-hydroxyl of **6** or **13** to the ketone (i.e. **3** and/or the *E*-isomer). Consistent with the proposed mechanism of inactivation of SAH hydrolase by **6**, **13**, and **14** was the observation that incubation of purified rat liver SAH hydrolase with **6** resulted in release of 1 equiv of fluoride ion (by ^{19}F NMR) and incubation with **14** resulted in release of 2 equiv of fluoride ion. The general synthetic route developed for the synthesis of the title nucleosides utilized the fluoro Pummerer reaction for the introduction of fluorine into the requisite precursors. Preliminary antiretroviral data from Moloney leukemia virus (MoLV) is presented and correlates with SAH hydrolase inhibition. Antiviral activity (IC_{50} against MoLV) ranged from 0.05 to 10 μg/mL.

S-Adenosyl-L-homocysteine (SAH) hydrolase (EC 3.3.1.1.) catalyzes the reversible hydrolysis of SAH (**1**) to adenosine and homocysteine (Scheme I).[1] The mechanism by which this enzymatic hydrolysis takes place has been determined[2,3] (see Scheme I). SAH hydrolase contains tightly bound NAD^+, which is reduced to NADH with concomitant oxidation of the 3'-hydroxyl group of the nucleoside and the subsequent formation of the 3'-keto-5'-methylene intermediate **2**. The equilibrium of the reaction favors synthesis, but the reaction proceeds in the hydrolytic direction because of enzymatic removal of adenosine and homocysteine.[1]

The inhibition of this enzyme has been correlated with antiviral activity[4] and has attracted considerable attention as a target for the discovery of new antiviral agents.[5] The inhibition of SAH hydrolase results in accumulation of SAH, a potent feedback inhibitor of *S*-adenosylmethionine (SAM) dependent transmethylation reactions.[1,6,7] For viral and eukaryotic mRNA, these methylations take place at N-7 of terminal guanosine residues and certain 2'-hydroxyl groups. This capping is required for efficient translation of viral mRNA into proteins.[8]

Several natural products or analogues thereof have been identified as inhibitors of SAH hydrolase.[9] One natural product that has received recent attention is neplanocin A,[10] whose mechanism of enzyme inhibition involves the tight binding of the 3'-keto form of the nucleoside to the

Scheme I



NADH form of SAH hydrolase.[11] Neplanocin A has broad antiviral activity[11,12] and antitumor activity against mouse leukemia L1210.[13] However, the inhibitor is rapidly deaminated to the inactive hypoxanthine analogue[14] and is metabolized by other enzymes.[15] Deoxy, arabino, and other analogues of neplanocin A have been synthesized,[16] but no reports of improved antiviral activity over neplanocin A have appeared. Other modified analogues, such

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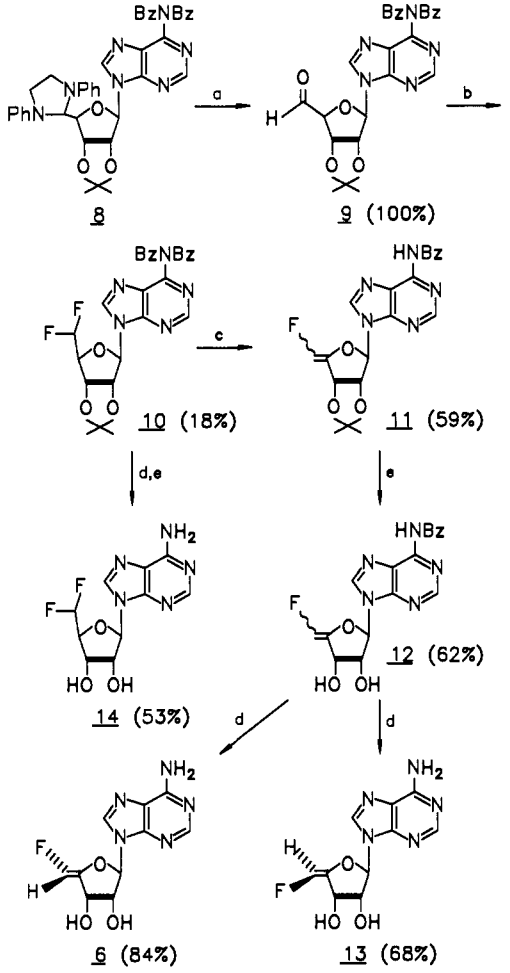
Scheme II[a]

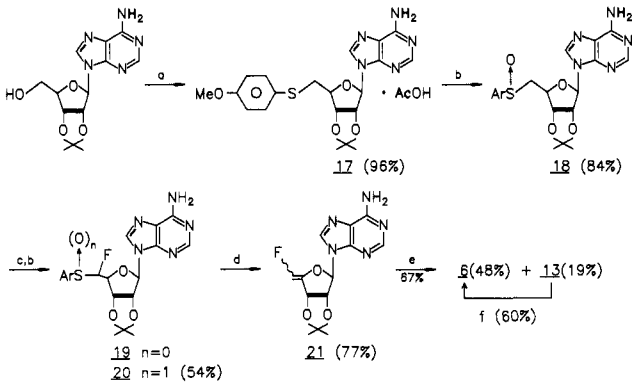


[a] Reagents: (a) $TsOH-H_2O$, CH_3COCH_3, CH_2Cl_2; (b) DAST, CH_2Cl_2; (c) KOtBu, DMSO; (d) EtOH, NH_3; (e) $CF_3COOH-H_2O$, 4:1. Bz = Benzoyl.

as 3-deazaneplanocin[17] and two analogues of neplanocin A, which lack the 5′-hydroxyl group,[18] are poor substrates for adenosine deaminase and potent inhibitors of SAH hydrolase.

Palmer and Abeles[7a] reported that 4′,5′-didehydro-5′-deoxyadenosine (**5**) is converted to both adenosine and SAH by SAH hydrolase, presumably via ketone **2**. This observation led us to design the corresponding vinyl fluorides **6** and **13** as potential mechanism-based inhibitors of SAH hydrolase.[19,20] As depicted in Scheme I for **6**, enzymatic oxidation would afford the potent electrophile **3**, which would be susceptible to addition by an enzyme nucleophile, followed by elimination of fluoride ion to yield

Scheme III[a]



[a] Reagents: (a) $(4-CH_3O-C_6H_4-S)_2$, Bu_3P, pyridine; (b) MCPBA, CH_2Cl_2; (c) DAST (Et_2NSF_3), $CHCl_3$; (d) $(CH_3OCH_2CH_2)_2O$, EtN(iPr)$_2$, 145 °C; (e) $CF_3CO_2H-H_2O$, 4:1; Bio-Rad AG1-X2(OH−), MeOH; (f) $h\nu$, acetone–CD_3OD.

inactivated enzyme. Alternatively, if the nucleophile were water, inactivation could still take place, since the product **4** (Nu = OH, equivalent to an aldehyde) may bind tightly to the NADH form of the enzyme or covalently attach to an active site residue through the 5′-aldehyde functionality. The latter type of binding has been suggested for adenosine dialdehyde.[21] In addition, we examined the possibility that 5′-deoxy-5′,5′-difluoroadenosine (**14**, Scheme I) may be a mechanism-based inhibitor of SAH hydrolase, forming the potent electrophile **3**, or its geometric isomer. In designing nucleosides **6**, **13**, and **14**, the lack of a 5′-hydroxyl group was anticipated to make these compounds poor substrates for adenosine deaminase.[22] Also, the 5′-fluorovinyl ether functional group was expected to impart greater acid stability relative to **5**.[23]

Synthesis

The first route to the target nucleosides, **6**, **13**, and **14**, is depicted in Scheme II. The starting material **8** was obtained from benzoylation of a known compound.[24] Deprotection yielded aldehyde **9**,[25] which was treated with (diethylamido)sulfur trifluoride (DAST) to provide difluoride **10** in 18% yield. Dehydrofluorination with KOtBu in DMSO yielded vinyl fluorides **11** as a 2:1 mixture of *Z*- and *E*-isomers. Concomitant loss of one benzoyl group was observed. Deprotection to diols **12** afforded separable isomers. These were individually converted to nucleosides **6** and **13**. Intermediate **10** was also deprotected to nucleoside **14** with trifluoroacetic acid followed by ammonia.

In view of the poor overall yield of this route, a new general approach to the vinyl fluoride nucleosides that could be applied to the synthesis of **34** and **35** was needed. Criteria for the new route were the lack of strong basic conditions and an improved yield in the fluorination step. Because the 4′,5′-didehydro nucleoside **5** has been synthesized from 5′-(phenylseleno)-5′-deoxyadenosine by oxidation and subsequent thermolysis at 100 °C,[26] we con-

(17) (a) Christopher, K. H. T.; Marquez, V. E.; Fuller, W. R.; Goldstein, B. M.; Haines, D. R.; McPherson, H.; Parsons, J. L.; Shannon, W. M.; Arnett, G.; Hollingshead, M.; Driscoll, J. S. *J. Med. Chem.* **1989**, *32*, 1442. (b) Glazer, R. I.; Knode, M. C.; Tseng, C. K. H.; Haines, D. R.; Marquez, V. E. *Biochem. Pharmacol.* **1986**, *35*, 4523.
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(19) A similar mechanism was recently reported for an inhibitor of D-xylose isomerase: Carrell, H. L.; Glusker, J. P.; Burger, V.; Manfre, F.; Tritsch, D.; Biellmann, J.-F. *Proc. Natl Acad. Sci. U.S.A.* **1989**, *86*, 4440.
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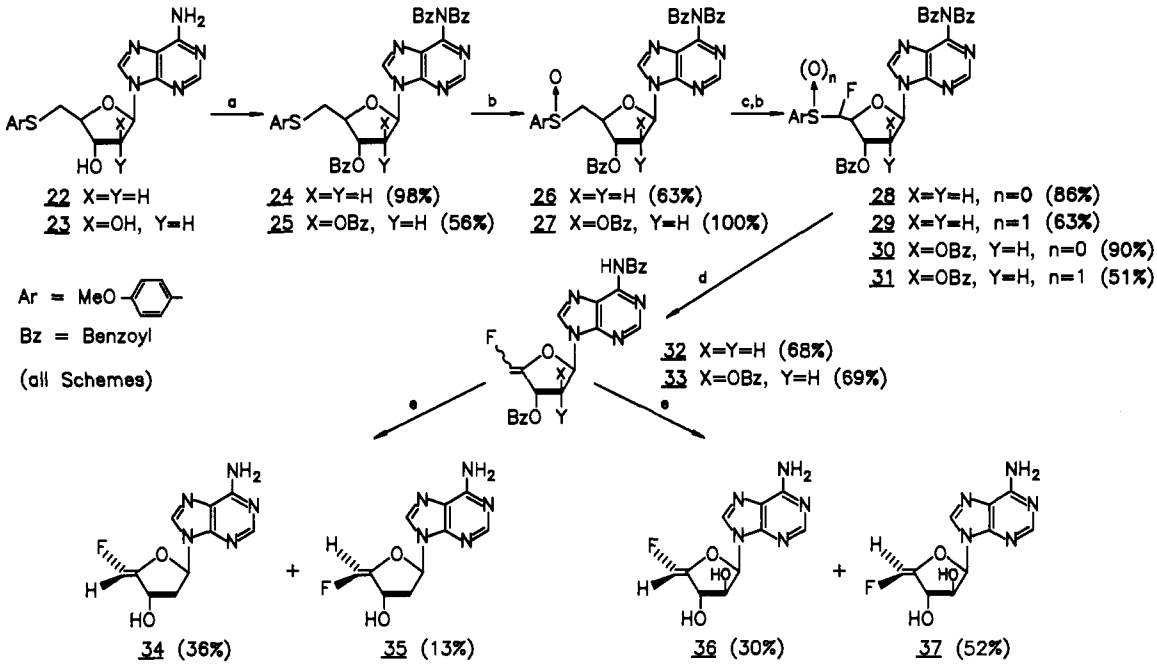
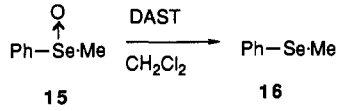
(25) Aldehyde **9** was reported as an intermediate in Vrudhula et al.: Vrudhula, V. M.; Kappler, F.; Afshar, C.; Ginell, S. L.; Lessinger, L.; Hampton, A. *J. Med. Chem.* **1989**, *32*, 885.
(26) Zylber, N.; Zylber, J.; Gaudemer, A. *J. Chem. Soc. Chem. Comm.* **1978**, 1084.

Scheme IV[a]



[a] Reagents: (a) Benzoyl chloride, pyridine; (b) MCPBA, CH_2Cl_2; (c) DAST, $ClCH_2CH_2Cl$; (d) xylene (for **32**) or diglyme (for **33**); (e) EtOH, NH_3.

sidered extending our fluoro-Pummerer method[27] to similar α-fluoro selenoxides. However, preliminary studies with phenyl methyl selenoxide (**15**) indicated that DAST exclusively deoxygenated **15** to phenyl methyl selenide (**16**) under the usual conditions.[27]



Therefore, we turned to the sulfoxide approach (Scheme III), with some concern for the high temperatures that might be required for the thermal elimination step. Isopropylideneadenosine was treated with *p*-anisylidine disulfide under the conditions of Nakagawa et al.[28] to provide thioether **17** in 96% yield as the crystalline acetic acid complex. No cyclonucleoside was observed, which is a major side reaction with dialkyl disulfides.[29] Oxidation of **17** with *m*-chloroperbenzoic acid (MCPBA) afforded the sulfoxides **18**. Either sulfoxide or the diastereomeric mixture was transformed to the same mixture of fluoro thioethers **19** with DAST. Further oxidation with MCPBA yielded sulfoxides **20** as a mixture of isomers. A recent paper by Robins and Wnuk[30] described similar transformations on nucleosides using $SbCl_3$ as a catalyst. In our case the *p*-anisylidine group allows the fluoro-Pummerer reaction to proceed without a catalyst. The *p*-anisylidine-containing intermediates were usually crystalline.

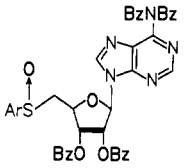
For the thermolysis of **20**, diglyme was the solvent of choice, providing good solubility and a high boiling point. The reaction was carried out in the presence of diisopropylethylamine[31] and gave a 77% yield of **21** as a 2.7:1 mixture of *Z*- and *E*-isomers. Deprotection of **21** provided the desired products **6** and **13**. These isomers were separated on a Dekker column[32] and were identical with products obtained by the method outlined in Scheme II. The *E*-geometric isomer **13** was irradiated with a sun lamp in acetone–methanol, which resulted in a 60–70% conversion to *Z*-isomer **6**. The configurations of **6** and **13** were proven by NOE experiments between H-3′ and the vinyl proton (see Experimental Section).

The combined overall yield of **6** and **13** in Scheme III was 18%, and this general route was applied to deoxy and arabino analogues (Scheme IV). For these transformations benzoyl-protecting groups were used for both hydroxy and amino groups, and the intermediates were more soluble in organic solvents. The thermal syn elimination of sulfoxides **29** and **31** resulted in concomitant loss of one benzoyl group, as was observed for **10** (in Scheme II). In the arabino series (starting with **23**), the *E*- and *Z*-isomers of vinyl fluoride **33** were separated on silica gel and converted individually to **36** and **37**. In the deoxy series, the *Z*-isomer **34** was crystalline, whereas isomer **35** was an oil. The initial sample of **34** was isolated as an acetic acid complex.

For the synthesis of chlorovinyl nucleoside **44** (Scheme V), the acetic acid complex **17** was converted to free **38**, to avoid side reactions in the benzoylation step. Dibenzoate **39** was oxidized to sulfoxide **40** and chlorinated with sulfuryl chloride[33] to **41**. It should be noted that the acetonide-protecting group was required, as the desired reaction did not take place with benzoate **45**.

Sulfoxide elimination from **41** proceeded in the same manner as examples in Scheme III and IV (**20**, **29**, and **31**), affording **42** as a 5:1 mixture of *Z*- and *E*-isomers, re-

(27) McCarthy, J. R.; Peet, N. P.; LeTourneau, M. E.; Inbasekaran, M. *J. Am. Chem. Soc.* **1985**, *107*, 735.
(28) Nakagawa, I.; Aki, K.; Hata, T. *J. Chem. Soc. Perkin 1* **1983**, 1315.
(29) Craig, G. W.; Moffatt, J. G. *Nucleosides Nucleotides* **1986**, *5*, 399.
(30) Robins, M. J.; Wnuk, S. F. *Tetrahedron Lett.* **1989**, *29*, 5729.
(31) Amine has been added in a sulfoxide elimination. For example, Babler, J. H.; Haack, R. A. *J. Org. Chem.* **1982**, *47*, 4801.
(32) Dekker, C. A. *J. Am. Chem. Soc.* **1965**, *87*, 4027.
(33) Tsuchihashi, G.; Ogura, K.; Iriuchijima, S.; Tomisawa, S. *Synthesis* **1971**, 89.



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spectively. Deprotection of **42** followed by crystallization afforded pure *Z*-isomer **43**. Ammonolysis of **43** provided the desired **44**.

Fluorination of **40** with DAST followed by MCPBA oxidation (Scheme V) afforded fluoro sulfoxide **47**. Chlorination of **47** with sulfuryl chloride provided chloro fluoro sulfoxide **48**. Thermal elimination of the sulfoxide formed *E*-isomer **49** exclusively. Deprotection of **49** yielded the desired **51** and photolysis of **51** gave a small sample of isomer **52**. With both geometric isomers in hand, structural assignments were made for **51** and **52** on the basis of the carbon–fluorine and hydrogen–fluorine coupling constants (see Experimental Section).

Enzyme Inhibition: Results and Discussion

Initial studies on compounds **6**, **13**, and **14** were performed with partially purified SAH hydrolase from mouse liver. Enzyme activity was assayed in the synthetic direction as described by Ragione and Pegg.[34] The pseudo-first-order loss of activity observed for *Z*-isomer **6** is plotted in Figure 1. Kinetic constants were determined according to the method of Kitz and Wilson[35] and are listed in Table I. With the mouse liver enzyme, **6** and **13** had similar values of k_{inact} (Table I), but the *Z*-isomer **6** is the more potent inactivator by comparison of k_{inact}/K_I (5.0×10^5 min^{-1} M^{-1} for **6** and 2.2×10^5 min^{-1} M^{-1} for **13**). Adenosine protected the mouse liver enzyme from inhibition by **6**. Inhibition by **6** was not reversed by extensive dialysis or by incubation of the inactivated enzyme at 37 °C for 90 min with 0.5 mM NAD$^+$. The difluoromethyl compound **14** was a less potent time-dependent inhibitor than **6** or **13**, exhibiting $k_{inact}/K_I = 6 \times 10^3$ min^{-1} M^{-1}. This was in contrast to Palmer and Abeles' results[2a] where 5′-deoxy-5′-fluoroadenosine was not a substrate or an inactivator of beef liver SAH hydrolase. As **14** showed nonlinear kinetics with this partially purified enzyme, its K_I was estimated from the initial slope. This nonlinearity may be the result of metabolism of the inhibitor, since linear kinetics were obtained for **14** with the purified rat liver enzyme (Table I). No time-dependent inhibition was observed with **17**, **34**, **35**,[36] **36**, **37**, or **51** with mouse liver SAH hydrolase.

To confirm the above results and to perform further mechanistic studies, SAH hydrolase from rat liver was purified.[37] This enzyme was assayed in the hydrolytic direction spectrophotometrically.[38] The kinetic constants (Table I) show that **6** was a slightly more potent inactivator ($k_{inact}/K_I = 1.2 \times 10^5$ min^{-1} M^{-1}) than **13** ($k_{inact}/K_I = 0.58 \times 10^5$ min^{-1} M^{-1}), and dramatically more potent than difluoronucleoside **14** ($k_{inact}/K_I = 4.1 \times 10^3$ min^{-1} M^{-1}).

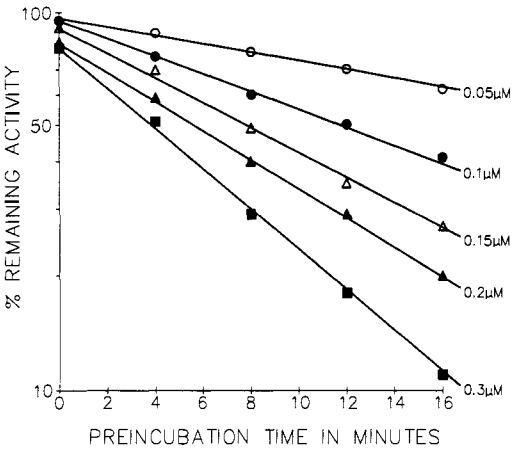

Figure 1. Time- and concentration-dependent inhibition of SAH hydrolase by **6**. Partially purified SAH hydrolase from mouse liver was incubated with various concentrations of **6**. At indicated times, aliquots were withdrawn and tested for remaining enzyme activity as described in the Experimental Section.

Compounds **34**, **36**, **37**, **44**, **51**, and **52** were competitive inhibitors and the K_i values were determined for the rat liver enzyme (Table I). Some interesting features of these competitive inhibitors should be pointed out, particularly those relating to the 5′-position. Assuming that K_i reflects binding affinity, the *E*-isomer **37** of the ara-A-derived nucleosides bound more tightly than the *Z*-isomer **36**, which is the order observed with time-dependent inhibitors **6** and **13** with this enzyme. Also, it is worthy of note that the chlorine substituent in **44**, **51**, and **52** does not prevent these nucleosides from binding to the active site of SAH. With the competitive inhibitors no NADH formation was observed during the course of the assay, suggesting that these inhibitors are not rapidly oxidized to the 3′-ketones. This difference in reactivity may have several reasons, but it is clear that substituents on the 5′-carbon play a significant role.

Within the series of inhibitors prepared, the 2′-hydroxyl group appears to be critical for time-dependent inhibition of SAH hydrolase as evidenced by the fact that nucleosides **34**, **35**, and **37** are not oxidized. This finding is of interest since ara-A and 2′-deoxyadenosine are oxidized by SAH hydrolase (and are time-dependent inhibitors)[1] despite the absence or stereochemical change of the 2′-hydroxyl. The 2′-hydroxyl is known to hydrogen bond to the 3′-hydroxyl, affecting its acidity, and has also been reported to affect ring conformation.[39] Nucleosides **34–37** contain modifications on both sides of the 3′-hydroxyl, which have unknown effects on such factors as the ring conformation and may explain the observation that these compounds are only competitive inhibitors.

Compounds **6** and **14** were deaminated by adenosine deaminase at least 1000-fold slower than adenosine. This slow deamination is consistent with the previously established substrate specificity of adenosine deaminase.[22]

Mechanistic Studies

Preliminary results[37] indicated that when **6** was mixed with purified (>90%) enzyme from rat liver stoichiometric formation of NADH was observed spectrophotometrically. After complete inactivation of the rat liver enzyme by **6**, the ^{19}F NMR spectrum of the supernatant obtained after denaturation and removal of the protein indicated that ca. 1 mol of fluoride ion was formed per mole of enzyme

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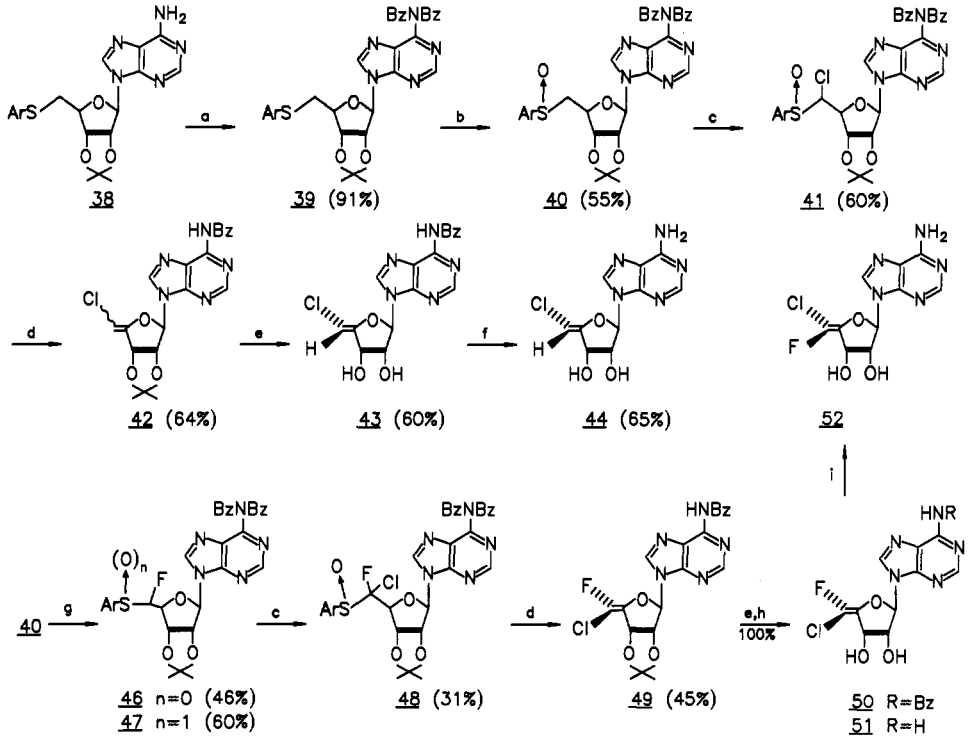
(35) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.
(36) Preincubation for 20 min with 5 μM **35** produced no time-dependent inhibition of the mouse enzyme. This compound was not further examined.
(37) Results with the rat liver enzyme have been briefly summarized.[20] Full details on mechanistic work with **6**, **13**, and **14** with this enzyme have been reported: Mehdi, S.; Jarvi, E. T.; Koehl, J. R.; McCarthy, J. R.; Bey, P. *J. Enzyme Inh.* **1990**, *4*, 1.
(38) Wolfson, G.; Chisholm, J.; Tashjian, A. H., Jr.; Fish, S.; Abeles, R. H. *J. Biol Chem.* **1986**, *261*, 4492.
(39) Sundaralingam, M. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 3.

Scheme V[a]



[a] Reagents: (a) PhCOCl, pyridine; (b) MCPBA, CH_2Cl_2; (c) SO_2Cl_2, CH_2Cl_2, pyridine; (d) diglyme, $EtN(iPr)_2$; (e) CF_3COOH–H_2O, 4:1; (f) MeOH, NH_3; (g) DAST, $CHCl_3$; (h) EtOH, NH_3; (i) $h\nu$, acetone, CH_3OH

Table I. Kinetic Constants for Enzyme Inhibition and IC_{50} Values against MoLV

	mouse liver SAH hydrolase[a]			rat liver SAH hydrolase[b]			
compd	time-dependent inhibition	K_I, μM	k_{inact}, min^{-1}	K_I, μM	k_{inact}, min^{-1}	K_i,[c] μM	IC_{50}, μg/mL MoLV[d]
6	yes	0.55	0.28	6.5	0.8	NA	0.05
13	yes	1.04	0.23	2.4	0.14	NA	0.25
14	yes	10[e]	0.6	9.7	0.04	NA	0.8
34	no	NA[g]	NA	NA	NA	150	10.0
35	no	NA	NA	NA	NA	ND[f]	ND
36	no	NA	NA	NA	NA	11.0	1.2
37	no	NA	NA	NA	NA	3.2	4.4
44	no	NA	NA	NA	NA	1.6	3.3
51	no	NA	NA	NA	NA	3.0	1.3
52	no	NA	NA	NA	NA	1.5	0.1
ara-A	yes	30	ND	ND	ND	NA	1.0

[a] Assayed in the synthetic direction (see Experimental Section). [b] Assayed in the hydrolytic direction (see Experimental Section). [c] K_I for competitive inhibition with rat liver SAH. [d] Method: see ref 42. [e] Value from initial slope. [f] ND = not determined. [g] NA = not applicable.

subunit. The stoichiometry indicates that the partition ratio (number of product molecules per each inactivation event) for inhibitor **6** is close to zero.

When **6** (tritiated in the 8-position) was incubated with the enzyme, labeled protein (0.8 mol of inhibitor/mol of enzyme subunit) was isolated. However, recent results[37] indicate that the label is lost under three different denaturing conditions.

The above experiments suggest that inhibitor **6** is oxidized to ketone **3**, which is then attacked by an enzyme nucleophile or water in the active site of the enzyme. If the displacement (addition–elimination) is rate-limiting in the inactivation, the *Z*-isomer would be predicted to be more potent than the *E*-isomer on the basis of kinetic results reported for the attack of nucleophiles on β-halo-α,β-unsaturated ketones.[40] It should be noted that the product is not reduced to the 3′-alcohol by the enzyme. If the product **4** contains a covalent link to the enzyme, it may be stable with respect to further degradation as long as the enzyme it not denatured. Alternatively, product **4** could be the addition product with water and remain tightly bound to the enzyme in the same manner as neplanocin A, which requires enzymatic oxidation. However, while inhibition of the beef liver enzyme by neplanocin A has been reported[41] to be reversed by incubation with NAD^+, addition of NAD^+ to SAH hydrolase (rat liver or mouse liver) inactivated by **6** did not reverse the inhibition. This suggests that the mechanism of inhibition by neplanocin A and **6** is different or that there is a difference between the enzymes from different sources.

The mechanism of inactivation by **14** was also investigated further with the rat liver enzyme.[37] Incubation of the enzyme with **14** led to formation of NADH as determined spectrophotometrically. When the enzyme was inhibited with an excess of **14**, examination of the ^{19}F spectrum of the supernatant revealed that the ratio of

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(41) Matuszewska; Borchardt, R. T. *J. Biol. Chem.* **1987**, *262*, 265.

moles of fluoride released to moles of enzyme subunits was 1.66, compared to roughly half that number in the case of inhibition by **6**. These results are consistent with the mechanism of inactivation of SAH hydrolase by **14** proposed in Scheme I.

Antiviral and Other Biological Activity

The nucleosides were evaluated as antiretroviral agents in the XC-plaque assay using Moloney leukemia virus (MoLV)[42] as a model for human immunodeficiency virus (HIV). The most potent antiviral activity was observed with time-dependent inhibitors **6** (IC_{50} = 0.05 μg/mL) and **13** (IC_{50} = 0.25 μg/mL). The less-potent inactivator **14** had antiviral activity in the same range as competitive inhibitors **44**, **36**, **37**, and **51**. In general, the results (Table I) indicate that enzyme inhibition correlates with antiviral activity. An exception to this trend is chloro fluoro nucleoside **52** which had antiretroviral activity in the same range as **6** and **13**. Aside from enzyme inhibition, other factors, such as membrane transport and cellular metabolism may affect antiviral activity. Among known SAH hydrolase inhibitors, ara-A has an IC_{50} of 1.0 μg/mL and neplanocin A has an IC_{50} of 0.008 μg/mL against MoLV. However, the latter compound is toxic and is metabolized to other derivatives.[15] The mechanism of antiviral activity was further investigated with **6**. MoLV infected fibroblasts (C3H10T1/2) were treated with 1.0 μg/mL of **6**. A 30% reduction in overall RNA methylation and a 88% reduction in constitutive MoLV production was observed[43] compared to controls. These results suggest that the inhibition of RNA-methylation may contribute to the antiretroviral activity at concentrations of **6** not toxic to these cells.

The most potent inactivator, **6**, was also evaluated against vaccinia and Herpes I virus. The observed IC_{50} values of 0.03 μM for vaccinia[44] and 10 μg/mL for Herpes I are in the range expected or SAH hydrolase inhibitors.[45] Finally, since SAH hydrolase inhibitors such as neplanocin A[44] exhibit cytotoxicity, nucleoside **6** was evaluated for HeLa cell antiproliferative activity. An IC_{50} of 1 μg/mL was measured, which is well above the antiviral IC_{50} values.

In summary, a new class of mechanism-based inhibitors of SAH hydrolase was designed, on the basis of the enzymatic pathway for the conversion of SAH to adenosine and homocysteine. Evidence was presented supporting the proposed mechanism of action outlined in Scheme I for vinyl fluoride nucleosides **6**, **13**, and the difluoro nucleoside **14**. Other analogues proved to be competitive inhibitors. Antiviral data against MoLV demonstrated that the time-dependent inhibitors **6** and **13** were potent in this model.

Experimental Section

General. All melting points are uncorrected. The IR spectra were recorded with a Perkin-Elmer Model 710B spectrophotometer, ^1H NMR spectra were determined with Varian EM-360A and Varian VXR-300 (multinuclear probe) spectrometers, and ^{19}F NMR spectra were recorded at 282 MHz on the Varian VXR-300 with $CFCl_3$ as an external standard. Mass spectra were obtained with a Finnigan MAT Model 4600 and are indicated as either electron impact (EI) or chemical ionization (CI). Exact mass determinations (HRMS) were obtained on a ZAB2-SE high-resolution mass spectrometer with perfluorokerosene as a reference. Combustion analyses for C, H, and N were performed by Merrell Dow Analytical Laboratories, Cincinnati, OH. Thin-layer chromatography (TLC) on silica gel plates was visualized by UV or by staining with alkaline potassium permanganate and heating. Reactions were carried out under nitrogen or argon, except for ammonia reactions and hydrolyses. Silica gel used for chromatography was 40–63 μM size, as described for flash chromatography.[46] Commercially available (Aldrich, Sure Seal) dry solvents were used as reaction media, unless otherwise indicated.

Caution: (Diethylamido)sulfur trifluoride (DAST) has been reported to decompose violently at temperatures above 50 °C.[47] Suitable safety precautions must be observed with this reagent. Reactions in this paper were carried out in an efficient fume hood in normal pyrex glassware, where some etching was observed.

Preparation and Assay of Mouse Liver SAH Hydrolase. SAH hydrolase was partially purified from mouse liver as described by Ragione and Pegg.[34] The 38–55% ammonium sulfate fraction was stored at −70 °C until use. The enzyme was assayed by measuring the radiolabeled *S*-adenosylhomocysteine formed from [2-^3H]adenosineand L-homocysteine. The assay mixture (200 μL) containing 0.025 mM potassium phosphate, 2 mM 2-mercaptoethanol, 0.1 mM adenosine, 0.28 μCi of [2-^3H]adenosine (21 Ci/mmol), 10 mM DL-homocysteine, 1.0 μM 2-deoxycoformycin, and enzyme was incubated at 37 °C for 10–20 min. The reaction was stopped by the addition of 0.8 mL of 30 mM HCl. A portion of the reaction mixture was applied to a column of Cellex-P (Bio Rad; 0.5 cm × 0.7 cm; H^+ form). The product, [2-^3H]SAH, was retained while adenosine was eluted quantitatively with 10 mL of 10 mM HCl. Radiolabeled SAH was then eluted with 2.0 mL of 0.5 M HCl and the radioactivity in the eluate was measured by liquid scintillation counting. By using this assay, kinetic constants for time-dependent inhibitors were determined by the method of Kitz and Wilson.[35]

Determination of Kinetic Constants for Rat Liver SAH Hydrolase. Rat liver SAH hydrolase was assayed by following the formation of homocysteine.[38] Assay conditions and preparation of the enzyme are given in the supplementary material. The K_i for a reversible inhibitor was determined from the initial rates of reactions containing varying concentrations of the inhibitor and using a correction for the [S] relative to the K_m. In one case (compound **36**), both inhibitor and substrate concentrations were varied, and a double reciprocal plot of the data was used to determine the type of inhibition and the K_i. For time-dependent inhibitors, the inactivation reaction was monitored continuously in the presence of substrate and inhibitor (**6**). The progress curves were fit to exponentials and the pseudo-first-order rate constants K_{obs} at different inhibitor concentrations were obtained. K_I and k_{inact} were obtained from a plot of $1/k_{obs}$ versus $1/[I]$. To test for reversibility of inhibition of the rat liver enzyme by compound **6**, a sample of inactivated enzyme was gel filtered to remove excess inhibitor, and incubated with NAD^+ (0.5 mM) or without NAD^+ at 37 °C for up to 90 min. Portions were removed to test for return of activity. A control sample of enzyme was incubated under the same conditions to monitor any effect on the activity.

^{19}F NMR Experiment with 6 and SAH Hydrolase from Rat Liver. In 0.82 mL of 1 mM EDTA and 10 mM potassium phosphate (pH 7.5), 0.46 mg (9.7 nmol of enzyme subunits) of SAH hydrolase was incubated with 75 nmol of **6**. After 10 min at room temperature, less than 2% of original enzyme activity was detected. Ethanol (2 mL) was added, the mixture was kept at 0 °C for 5 min and then centrifuged. The pellet was washed once with H_2O–EtOH and the combined supernatant and washing was taken to dryness in vacuo. The residue was dissolved in D_2O. Some insoluble material, possibly denatured protein, was present and was removed by centrifugation. The ^{19}F NMR spectrum (vs ext $CFCl_3$) showed signals due to fluoride (δ − 120.7) and unreacted inhibitor (δ − 162.5) in a 1:5.9 ratio. The ratio of enzyme to

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(43) Full details will be reported in a communication: Prakash, N. J.; Davis, G. F.; Jarvi, E. T.; McCarthy, J. R.; Bowlin, T. L. *Antiviral Res.* **1991**, in press.
(44) Kinney, M., Unpublished observations, Merrell-Dow, Indianapolis, IN.
(45) (a) Hasobe, M.; McKee, J. G.; Borcherding, D. R.; Borchardt, R. T. *Antimicrob. Agents Chemother.* **1987**, *31*, 1849. (b) De Clercq, E.; Cools, M.; Balzarini, J.; Marquez, V. E.; Borcherding, D. R.; Borchardt, R. T. *Antimicrob. Agents Chemother.* **1989**, *33*, 1291.
(46) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
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inhibitor used in the experiment was 1:7.1. Therefore, the amount of fluoride was approximately stoichiometric with the amount of enzyme used. No other signals were observed.

The above experiment was run under routine ^{19}F NMR conditions. The ^{19}F NMR spectrum of a mixture of **6** and fluoride ion was repeated with a 22 s pulse delay. Ratios of integrals were within 10% of those under routine ^{19}F NMR conditions.

Deamination by Adenosine Deaminase. Decrease in absorbance at 265 nm ($-\Delta\epsilon = 8.6$ mM^{-} cm^{-1}) due to the deamination of adenosine and of compounds **6** and **14** was monitored spectrophotometrically. The reaction mixture contained adenosine or the adenosine derivative (50 μM) and calf spleen or intestinal mucosa adenosine deaminase in 20 mM potassium phosphate (pH 7.5).

Chemistry. N^6,N^6-Dibenzoyl-5′-deoxy-2′,3′-*O*-isopropylidene-5′,5′-(*N*,*N*′-diphenylethylenediamino)adenosine (8). N^6-Benzoyl-5′-deoxy-2′,3′-*O*-isopropylidene-5′,5′-(*N*,*N*′-diphenylethylenediamino)adenosine[24] (2.96 g, 4.9 mmol) in pyridine (10 mL) was cooled in an ice bath, and benzoyl chloride (1.15 mL, 9.9 mmol) was added. The mixture was stirred overnight at room temperature and poured into ice water. The product was extracted with $CHCl_3$ (100 mL) and dried ($MgSO_4$). The solution was evaporated and toluene was added. Evaporation was repeated followed by drying in vacuo providing 4.07 g of a yellow foam. The product was percolated through a flash silica gel column (10 cm × 4 mm) with 4% EtOAc–96% CH_2Cl_2. Appropriate fractions were combined and evaporated three times from ethanol, providing a solid which was triturated with 50 mL of ethanol and filtered. The solid obtained was dried in vacuo, providing 2.67 g (76%) of **8**: mp 135–138 °C; ^1H NMR ($CDCl_3$) δ 1.32 (s, 3 H), 1.51 (s, 3 H), 3.48–3.71 (m, 4 H, NCH_2CH_2N), 4.61 (m, 1 H, H-4′), 5.14 (m, 2 H, H-2′, H-3′), 5.72 (d, 1 H, J = 2.6 Hz, N-C*H*-N), 6.12 (d, 1 H, J = 2.1 Hz, H-1′), 6.64 (d, 2 H, J = 7.9 Hz), 6.70–6.82 (m, 4 H), 7.13–7.27 (m, 4 H), 7.36 (t, 4 H, J = 7.6 Hz), 7.49 (m, 2 H), 7.87 (d, 2 H, J = 7.0 Hz), 7.95 (s, 1 H), 8.47 (s, 1 H); MS (CI/CH_4) 708 $(M + H)^+$. Anal. ($C_{41}H_{37}N_7O_5$) C, H, N.

N^6,N^6-Dibenzoyl-2′,3′-*O*-isopropylideneadenosine 5′-Aldehyde (9). N^6,N^6-Dibenzoyl-5′-deoxy-2′,3′-*O*-isopropylidene-5′,5′-(*N*,*N*′-diphenylethylenediamino)adenosine (**8**) (2.64 g, 3.73 mmol) in CH_2Cl_2 (370 mL) at 0 °C was added to a solution of *p*-toluenesulfonic acid monohydrate (1.56 g, 8.2 mmol) in 180 mL of acetone. The mixture was stirred for 1.5 h and filtered. The filtrate was evaporated and partitioned between 200 mL of CH_2Cl_2 and water. The CH_2Cl_2 solution was dried ($MgSO_4$) and evaporated to a foam. The foam (2.10 g) was dissolved in benzene (200 mL) and water was removed by refluxing with a Dean–Stark trap for 1 h. The solvent was removed in vacuo to provide 2.06 g (100%) of the title compound as a foam.

This material can be used for the following reaction, but the material used below (for best yields) was chromatographed on flash silica gel with 15% EtOAc–85% CH_2Cl_2 and treated in a Dean–Stark setup as above, (80% recovery). The NMR indicated aldehyde (65%) and hydrate (35%) form, resulting in doubling of some NMR signals. Aldehyde **9** has been previously described as an intermediate for which no spectral data was reported:[25] ^1H NMR ($CDCl_3$) δ 1.31 (s, 3 H), 1.52 (s, 3 H), 4.61 (d, 1 H, J = 1.7 Hz or two s), 5.25 (d, 1 H, J = 5.9 Hz), 5.42 (dd, 1 H, J = 1.7 and 7.9 Hz), 6.17 (s, 1 H), 7.24–7.77 (several m, 10 H), 8.07 (s, 1 H), 8.44 (major) and 8.54 (minor, two s, both H-8), 9.26 (s, 0.65 H, CHO); MS (FAB-XENON) m/z 514 $(M + H)^+$. Anal. Calcd ($C_{27}H_{23}N_5O_6$) C, 63.15; H, 4.51; N, 13.64. Found: C, 64.48; H, 5.08; N, 11.63.

N^6,N^6-Dibenzoyl-5′-deoxy-5′,5′-difluoro-2′,3′-*O*-isopropylideneadenosine (10). N^6,N^6-Dibenzoyl-2′,3′-*O*-isopropylideneadenosine 5′-aldehyde (**9**) (3.90 g, 7.6 mmol) was dissolved in 25 mL of CH_2Cl_2 (distilled from calcium hydride) and 3.2 mL (22.8 mmol) of (diethylamido)sulfur trifluoride was added. The mixture was stirred for 6 h, diluted with $CHCl_3$, and poured into saturated aqueous $NaHCO_3$ (50 mL). The product was extracted into chloroform (400 mL). The solution was dried ($MgSO_4$) and evaporated to a foam (3.60 g). The foam was percolated through a silica gel flash column (12 cm × 40 mm) with 4% EtOAc–96% CH_2Cl_2. The component of R_f 0.6 (10% EtOAc–90% CH_2Cl_2 TLC solvent) was isolated as a white foam (738 mg, 18%): ^1H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 3 H), 1.65 (s, 3 H), 4.42–4.53 (m, 1 H), 5.27 (dd, 1 H, J = 2.7 and 5.9 Hz), 5.39 (dd, 1 H, J = 1.7 and 6.0 Hz), 5.96 (td, 1 H, J = 55 and 4.5 Hz), 6.28 (d, 1 H, J = 1.3 Hz), 7.34–7.52 (m, 6 H), 7.85 (d, 4 H, J = 7.2 Hz), 8.15 (s, 1 H), 8.67 (s, 1 H); ^{19}F NMR ($CDCl_3$) δ −65.87 (ddd, J = 12.4, 55.2 and 299.0 Hz); −50.71 (ddd, J = 10, 55.2 and 299.1 Hz); MS (FAB-XENON) m/z 536 $(M + H)^+$. Anal. Calcd. ($C_{27}H_{23}F_2N_5O_5$) C, 60.56; H, 4.33; N, 13.08. Found C, 60.26; H, 4.44; N, 12.03.

5′-Deoxy-5′,5′-difluoroadenosine (14). The N^6,N^6-dibenzoylated **10** (407 mg, 0.76 mmol) was dissolved in absolute ethanol and evaporated to dryness. The residue was treated with ethanolic ammonia (15 mL, saturated at 0 °C) in a Carius tube for 24 h and evaporated in vacuo. The residue was purified by flash chromatography (1:1 EtOAc–CH_2Cl_2 followed by EtOAc), providing 195 mg (78%) of 5′-deoxy-5′,5′-difluoro-2′-3′-*O*-isopropylidineadenosine, (R_f = 0.21, EtOAc, TLC). A sample was triturated with CH_2Cl_2–cyclohexane to afford crystals, mp 161–163 °C. The crude product was used directly in the next step: ^1H NMR ($CDCl_3$) δ 1.42 (s, 3 H), 1.64 (s, 3 H), 4.37–4.47 (m, 1 H), 5.30–5.36 (m, 1 H), 5.42–5.44 (m, 1 H), 5.68 (bs, 2 H, NH_2), 6.01 (td, 1 H, J = 56 and 5.2 Hz), 6.19 (m, 1 H, coveredby 6.01 td), 7.87 (s, 1 H), 8.36 (s, 1 H); ^{19}F NMR −125.77 (ddd, J = 10.7, 55.8, and 259 Hz), −130.16 (ddd, J = 10.7, 55.8, and 259 Hz); MS (EI) m/z 327 (M^+); HRMS calcd for $C_{13}H_{15}N_5O_3F_2$ 327.1143, found 327.1140. Anal. ($C_{13}H_{15}N_5O_3F_2$) C, H, N.

To 5′-deoxy-5′,5′-difluoro-2′,3′-*O*-isopropylideneadenosine (165 mg, 0.76 mmol) was added 2 mL of 4:1 CF_3COOH–water. The mixture was stirred for 1 h and evaporated. The residue was purified by flash chromatography (200 mL of EtOAc followed by 400 mL of 5% MeOH–95% EtOAc). The appropriate fractions were combined and evaporated to afford 153 mg (100%) of a white foam (R_f = 0.35, 10% MeOH–90% EtOAc). The foam was stirred in 4 mL of ethanol and the resulting crystals were filtered to give 52 mg (36%) of **14**, mp 198–200 °C after drying in vacuo. Thermogravimetric analysis indicated 6% weight loss up to 200 °C and the only impurity found in the assayed sample was ethanol: ^1H NMR (DMSO-d_6, ethanol peaks excluded) δ 4.03–4.12 (m, 1 H), 4.36 (m, 1 H), 4.77 (q, 1 H, J = 6 Hz), 5.67 (d, 1 H, J = 5.1 Hz, OH), 5.70 (d, 1 H, J = 6.3 Hz, OH), 6.13 (d, 1 H, J = 6.3 Hz), 6.31 (td, 1 H, J = 53 and 4.7 Hz), 7.34 (b s, 2 H, NH_2), 8.16 (s, 1 H), 8.32 (s, 1 H); ^{19}F NMR (DMSO-d_6) −125.9 (ddd, J = 9.3, 55.1, and 291.2 Hz), −127.2 (ddd, J = 9.9, 54.9, and 291.2 Hz); MS (CI/CH_4) m/z 288 $(M + H)^+$; HRMS calcd for $C_{10}H_{11}O_3N_5F_2$ 287.0830, found 287.0850. The ethanol filtrate which contained CF_3CO_2H was evaporated and dissolved in water. A Dowex 50 (H^+) resin (H_2O, then 2 M NH_4OH) provided an additional 47 mg (total yield 68%) of **14**.

N^6-Benzoyl-9-(5-deoxy-5-fluoro-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (11). To 401 mg (0.75 mmol) of crushed N^6,N^6-dibenzoyl-5′-deoxy-5′,5′-difluoro-2′,3′-*O*-isopropylideneadenosine (**10**) and 335 mg (4 equiv) of potassium *tert*-butoxide under nitrogen was added 2 mL of dimethyl sulfoxide (distilled from CaH_2 and stored over molecular sieves). The mixture was stirred under nitrogen for 21 h, quenched with 4 mL of saturated ammonium chloride and extracted with ethyl acetate. The ethyl acetate layer afforded 274 mg of yellow oil which was percolated through a flash column (20 mm × 15 cm) with 30% EtOAc–70% CH_2Cl_2. TLC revealed two spots very close together at R_f = 0.55 (EtOAc solvent). Combined fractions yielded 183 mg (59%) of a foam containing the *Z*- and *E*-isomers in a 2:1 ratio: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.34 (major) and 1.37 (minor, 3 H together, 2 s), 1.49 (s, 3 H), 5.35–5.38 (m, 1 H), 5.56 and 5.90 (1 H together; d, J = 4 Hz and m, respectively), 6.23 (br s, minor) and 6.25 (1 H together), 6.43 and 6.81 (d, J = 74 Hz, major and d, J = 77 Hz, minor; 1 H together), 7.39–7.98 (m, 6 H), 8.65 (major) and 8.653 (minor; 2 s, 1 H together), 9.05 (1 H, br, NH); ^{19}F NMR ($CDCl_3$, 282 MHz) δ (from external $CFCl_3$) −158.94 (d, J = 74 Hz, major), 174.4 (d, J = 77 Hz, minor); MS (CI/CH_4) m/z 412 $(M + H)^+$; HRMS calcd for $C_{20}H_{18}FN_5O_4$ 411.1343, found 411.1330.

N^6-Benzoyl-9-(5-deoxy-5-fluoro-β-D-*erythro*-pent-4-enofuranosyl)adenine (12). Cold 4:1 CF_3COOH–water (10 mL) was used to dissolve 1.28 g (3.1 mmol) of N^6-benzoyl-9-(5-deoxy-5-fluoro-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)-adenine (**11**), and the mixture was stirred for 1 h at room temperature. The reaction was evaporated in vacuo and methanol was added and evaporated, giving 1.60 g of a foam. The foam was percolated through a 40-mm-diameter 17-cm flash silica gel

column consecutively with EtOAc, 5% MeOH–95% EtOAc, and 10% MeOH–90% EtOAc, affording 394 mg of the *E*-isomer (R_f = 0.47 with 10% MeOH–90% EtOAc), 80 mg of a mixture of *E*- and *Z*-isomers and 762 mg of the *Z*-isomer (R_f = 0.37 with 10% MeOH–90% EtOAc). Each of these fractions contained CF_3COOH by ^{19}F NMR. The 762 mg of *Z*-isomer was stirred in 3 mL of 10% MeOH–90% EtOAc, filtered, and dried to give 491 mg (42%) of the *Z*-isomer of **12** free of CF_3COOH. The partially purified *E*-isomer from above (394 mg) was similarly treated, affording 230 mg (20% yield) of the *E*-isomer of **12** free of CF_3COOH.

(*Z*)-**12**: mp 150–158 °C; ^1H NMR (CD_3OD, 90 MHz) δ 5.1 (m, 2 H), 6.35 (d, 1 H, J = 6 Hz), 7.5–8.2 (m, 5 H), 8.63 (s, 1 H), 8.72 (s, 1 H); HRMS calcd for $C_{17}H_{14}FN_5O_4$ 371.1030, found 371.1015.

(*E*)-**12**: mp 155–160 °C; ^1H NMR (CD_3OD, 90 MHz) δ 5.00–5.10 (m, 2 H), 6.37 (1 H, d, J = 7 Hz), 6.48 (1 H, d, J = 7 Hz), 6.48 (1 H, Cl, J = 75 Hz), 7.54–8.19 (m, 5 H), 8.53 (s, 1 H), 8.62 (s, 1 H); HRMS calcd for $C_{17}H_{14}FN_5O_4$ 371.1030, found 371.1028.

(*Z*)-9-(5-Deoxy-5-fluoro-β-D-*erythro*-pent-4-enofuranosyl)adenine (6). Method A. A suspension of 491 mg (1.6 mmol) of (*Z*)-**12** in (ca. 10 mL) EtOH was evaporated. The residue in EtOH (35 mL) was saturated with anhydrous ammonia at 0 °C. The resulting solution was capped (Carius tube) and stirred at room temperature for 18 h. The mixture was evaporated and the residue recrystallized from methanol (6 mL) to afford 217 mg (61% yield) of **6** (dried under high vacuum at 80 °C). The filtrates were chromatographed (30 mm × 17 cm flash silica gel) with 10% MeOH–90% EtOAc to give an additional 83 mg (84% combined yield) of **6**, identical in all respects with the material prepared by Method B from **21**.

(*E*)-9-(5-Deoxy-5-fluoro-β-D-*erythro*-pent-4-enofuranosyl)adenosine (13). Method A. A suspension of (*E*)-**12** (103 mg, 0.33 mmol) in 10 mL of EtOH was evaporated and the residue treated with ethanolic ammonia as described for (*Z*)-**12**. The residue from evaporation of the ethanolic ammonia was stirred in 1 mL of MeOH and filtered to provide 51 mg of **13** as a white solid (68% yield), identical with the product prepared by Method B from **21**.

5′-Deoxy-5′-[(4-methoxyphenyl)thio]-2′,3′-*O*-isopropylideneadenosine (17) (AcOH Complex). To a mixture of 2′,3′-*O*-isopropylideneadenosine (16.4 g, 0.053 mol) and 4-methoxyphenyl disulfide (26.0 g, 0.094 mol) (Fairfield Chemical) in dry pyridine (125 mL) was added tributylphosphine (23.3 mL, 0.094 mol) via syringe. After 15 min at room temperature, a solution formed. The reaction was stirred overnight under argon, diluted with an equal volume of ethyl acetate, and washed with cold 0.5 N NaOH (2 × 50 mL), dilute acetic acid (2 × 50 mL), and brine (50 mL). The organic layer was dried ($MgSO_4$) and evaporated to an oil that crystallized under high vacuum. The white crystals were triturated with hexane (2 × 500 mL) and dried in a vacuum oven at 50 °C, providing **17** as an analytically pure acetic acid complex (24.9 g, 96%): mp 70 °C to a wax that clears at 97 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.32 (s, 3 H), 1.50 (s, 3 H), 1.91 (s, 3 H, HOAc), 3.07 (dd, 1 H, J = 13.7 and 6.5 Hz), 3.15 (dd, 1 H, J = 13.9 and 8.2 Hz), 3.74 (s, 3 H), 4.14 (ddd, 1 H, J = 2.3, 6.9, and 6.4 Hz), 5.04 (dd, 1 H, J = 6.1 and 2.5 Hz), 5.52 (dd, 1 H, J = 6.1 and 2.2 Hz), 6.16 (d, 1 H, J = 2.0 Hz), 6.88 (d, 2 H, J = 8.8 Hz), 7.31 (d, 2 H, J = 8.6 Hz), 8.17 (s, 1 H), 8.33 (s, 1 H); MS (CI/CH_4) m/z 430 (M + H)$^+$. Anal. ($C_{20}H_{23}N_5O_4S\cdot CH_3CO_2H$) C, H, N.

5′-Deoxy-5′-[(4-methoxyphenyl)sulfinyl]-2′,3′-*O*-isopropylideneadenosine (18). To a solution of **17** (23.1 g, 0.054 mol) in CH_2Cl_2 (225 mL) cooled to −40 °C was added a solution of 85% 3-chloroperbenzoic acid (11.1 g, 0.054 mol) in CH_2Cl_2 (225 mL) dropwise. The reaction was allowed to warm to room temperature, washed with ice cold saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated in vacuo. The resulting gel was purified by flash silica gel chromatography (1 L). Elution with ethanol–ethyl acetate (5:95) provided sulfone (2.2 g), IR (KBr) 1140 cm^{-1}. Further elution with ethanol–EtOAc (1:9) provided the diastereomeric sulfoxides, eluting as two bands. The fractions containing product were combined and evaporated to a white solid (20.1 g, 84%). In another run, the sulfoxides were isolated separately in a 10:9.2 ratio (faster moving:slower moving by TLC). Faster moving sulfoxide: IR (KBr) 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.37 (s, 3 H), 1.61 (s, 3 H), 3.16 (dd, 1 H, J = 13.1 and 4.0 Hz), 3.33 (dd, 1 H, J = 13.1 and 10.2 Hz), 3.83 (s, 3 H), 4.80 (dd, 1 H, J = 4.5 and 3.3 Hz), 5.09 (dd, 1 H, J = 6.2 and 3.3 Hz), 5.55 (dd, 1 H, J = 6.2 and 1.8 Hz), 5.78 (br s, 2 H), 6.08 (d, 1 H, J = 1.8 Hz), 6.92 (d, 2 H, J = 8.9 Hz), 7.37 (d, 2 H, J = 9.0 Hz), 7.87 (s, 1 H), 8.26 (s, 1 H); MS (CI/CH_4) m/z 446 (M + H)$^+$; HRMS calcd for $C_{20}H_{23}N_5O_5S$ 445.1420, found 445.143. Slower moving sulfoxide: IR (KBr) 1089 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.37 (s, 3 H), 1.57 (s, 3 H), 3.27 (dd, 1 H, J = 13.3 and 5.7 Hz), 3.38 (dd, 1 H, J = 13.6 and 7.1 Hz), 3.84 (s, 3 H), 4.55 (ddd, 1 H, J = 2.8, 6.4 and 6.4 Hz), 5.27 (dd, 1 H, J = 6.3 and 2.9 Hz), 5.35 (dd, 1 H, J = 6.5 and 2.3 Hz), 5.51 (dd, 1 H, J = 6.3 and 2.4 Hz), 5.73 (br s, 2 H), 6.05 (d, 1 H, J = 2.6 Hz), 6.93 (d, 2 H, J = 8.7 Hz), 7.48 (d, 2 H, J = 8.8 Hz), 7.90 (s, 1 H), 8.33 (s, 1 H); HRMS calcd for $C_{20}H_{23}N_5O_5S$ 445.1420, found 445.1416.

5′-Deoxy-5′-fluoro-5′-[(4-methoxyphenyl)sulfinyl]-2′,3′-*O*-isopropylideneadenosine (20). Sulfoxide **18** (as a mixture of diastereomers) (10.5 g, 0.0236 mol) was dissolved in CH_2Cl_2 (150 mL) and (diethylamido)sulfur trifluoride (13.2 mL, 0.1 mol) was added all at once. The reaction was immediately placed in a preheated oil bath (45 °C) and stirred under an argon atmosphere. The progress of the reaction was followed by TLC ($CHCl_3$–acetone, 1:1) and after 2.5 h, the solution was washed with ice cold saturated aqueous $NaHCO_3$, dried (K_2CO_3), and cooled to 10 °C with an ice bath. [A small aliquot of **19** was evaporated in vacuo for ^{19}F NMR ($CDCl_3$, 282 MHz) δ −159.14 (dd, J = 53.3 and 14.4 Hz), −159.79 (dd, J = 53.0 and 13.8 Hz). The integrated peak ratios were 10:4.2 for the two fluoro sulfides and the product ratios were the same starting with either isomer of the sulfoxide or the diastereoisomeric mixture.] The solution was stirred and treated portionwise with solid 85% 3-chloroperbenzoic acid (8.5 g) until TLC ($CHCl_3$–acetone, 1:1) indicated complete conversion to sulfoxide. The reaction solution was washed with ice cold saturated aqueous $NaHCO_3$, dried (K_2CO_3), and evaporated to a tan foam. The foam was purified by flash chromatography (700 mL silica gel) ($CHCl_3$–acetone 3:2) providing **20** as a light tan solid (5.9 g, 54%) as a mixture of diastereomers: mp 106–114 °C; IR (KBr) 1180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4–1.65 (several s, 6 H), 3.81–3.86 (singlets, 3 H, MeO), 4.4–5.8 (several m, 4 H), 6.0–6.3 (several m, 3 H), 6.65–8.36 (several m and s, 6 H); ^{19}F NMR ($CDCl_3$) δ −193.11 (dd, J = 47.7 and 26.8 Hz) and −195.05 (dd, J = 47.7 and 19.5 Hz) for two major diastereoisomers; MS (CI/CH_4) m/z 464 (M + H)$^+$; HRMS calcd for $C_{20}H_{22}FN_5O_5S$ 463.13257, found 463.1317.

(*E*)- and (*Z*)-4′,5′-Didehydro-5′-deoxy-5′-fluoro-2′,3′-*O*-isopropylideneadenosine (21). A solution of fluoro sulfoxide **20** (6.5 g, 0.014 mol), diglyme (120 mL), and diisopropylethylamine (9 g, 0.07 mol) was purged with nitrogen for 20 min and then heated at 145 °C (bath temperature). The progress of the reaction was followed by TLC (EtOAc–MeOH 9:1). After 24 h an additional 6 g of diisopropylethylamine was added and the reaction was heated an additional 24 h. The solvent was removed by Kugelrohr distillation under high vacuum at ca. 70–80 °C, and the residue was purified by flash chromatography (700 mL of silica gel; EtOAc–MeOH 96:4) to provide 3.3 g (77%) of **21** as a white solid, which was a 2.7:1 mixture of *Z*- and *E*-isomers: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.44 and 1.47 (2 s, 3 H, *E*- and *Z*-isomer), 1.59 (s, 3 H), 5.49 (t, 1 H, J = 6.4 Hz), 5.66 (m, 3 H, includes NH_2), 6.27 and 6.28 (2 d, 1 H, J = 1.4 and 0.9 Hz, *E*- and *Z*-isomer), 6.51 and 6.91 (2 d, J = 74.2 and 77.2 Hz, *Z*- and *E*-isomers, respectively), 7.81 and 7.85 (2 s, 1 H, *E*- and *Z*-isomer), 8.31 and 8.33 (2 s, 1 H, *Z*- and *E*-isomer); ^{19}F NMR ($CDCl_3$, 282 MHz) δ (from $CFCl_3$) −160.21 (d, J = 74.5 Hz), −175.66 (d, J = 77.3 Hz); integrated ratio 3.4 to 1.0; ms (CI/CH_4) m/z 308 (M + H)$^+$; HRMS calcd for $C_{13}H_{14}FN_5O_3$ 308.1159, found 308.1161.

(*Z*)- and (*E*)-(5-Deoxy-5-fluoro-β-D-*erythro*-pent-4-enofuranosyl)adenine (6) and (13). Method B. The protected vinyl fluoride **21** (3.2 g, 0.01 mol) was dissolved in CF_3COOH–water (75:25) (130 mL) and stirred at room temperature for 16 h. The solution was evaporated to dryness in vacuo at room temperature and reevaporated several times with ethanol. The residue was dried under high vacuum at room temperature (4.77 g) and dissolved in methanol (100 mL). The solution was divided into four 25-mL aliquots and applied to four BIO-RAD AD 1-X2 (OH^- form) (200–400 mesh) columns (200 mL of resin each) packed with methanol.[32] The columns were eluted with methanol

at a rate of 4 mL/min and fractions were monitored by a UV detector. After 3 days, the faster moving *E*-isomer (**13**) started to elute from the column. After 14 h, the peak had completely eluted and the *Z*-isomer (**6**) started to elute and was collected in ca. 1.4 L of eluant. The two separate bands were concentrated to white crystalline solids in vacuo providing 0.50 g (19%) of the faster moving **13** and 1.30 g (48%) of the slower moving **6**. A small sample of **6** was recrystallized from EtOAc: mp 218–221 °C dec; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 4.60 (t, 1 H, J = 4.6 Hz), 5.05 (dd, 1 H, J = 6.9 and 4.8 Hz), 5.64 (d, 1 H, J = 4.6 Hz, OH), 5.79 (d, 1 H, J = 6.6 Hz, OH), 6.22 (d, 1 H, J = 7.1 Hz), 6.67 (d, 1 H, J = 75.8 Hz), 7.38 (s, 2 H, NH_2), 8.17 (s, 1 H), 8.46 (s, 1 H); ^{19}F NMR (CD_3OD, 282 MHz) δ (from external $CFCl_3$) −166.76 (d, J = 75.2 Hz); MS (CI/CH_4) m/z 268 $(M + H)^+$ $[\alpha]^{20}_D$ = −59.4° (MeOH). Anal. ($C_{10}H_{10}FN_5O_3$) C, H, N.

A small sample of **13** was recrystallized from acetone: mp 233–234 °C dec; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 4.68 (m, 1 H), 5.05 (m, 1 H), 5.78 (m, 1 H, OH), 7.15 (d, 1 H, J = 78.5 Hz), 7.38 (m, 2, NH_2), 8.16 (s, 1 H), 8.45 (s, 1 H); ^{19}F NMR (DMSO-d_6) δ (from external $CFCl_3$) −181.46 (d, J = 78.7 Hz); MS (CI/CH_4) m/z 268 $(M + H)^+$. Anal. ($C_{10}H_{10}FN_5O_3$) C, H, N.

NOE difference spectroscopy (DMSO-d_6) on **6** and **13** showed a 5% enhancement in the vinyl proton on **6** (δ 6.67) when the 3′-methine proton (δ 4.60) was irradiated and no enhancement on the vinyl proton on **13** (δ 7.15) when the 3′-methine proton (δ 4.68) was irradiated. The coupling constants from C-3′ to the fluorine were also measured, and J_{C-F} is 4.8 Hz for **6** and 0 for **13**.

Conversion of 13 to 6. Vinyl fluoride **13** (20 mg, 0.075 mmol) was dissolved in 3 mL of acetone-CD_3OD (2:1) and placed in an NMR tube. The solution was irradiated with a 275-W sunlamp placed 19 cm from the tube. The progress of the conversion was followed by ^{19}F NMR. After 4.5 h, conversion was greater than 60%. Prolonged irradiation caused decomposition. The solution was concentrated, redissolved in 5 mL of CH_3OH and purified by passage through 5 mL of BIO-RAD AG 1-X2 (OH form) (200–400 mesh) (CH_3OH) to give 12 mg (60%) of **6** and 8 mg (40%) of **13**.

Experimental details for compounds **22**, **24**, **26**, **28**, **29**, and **32** are given in the supplementary material.

(*Z*)- and (*E*)-4′,5′-Didehydro-2′,5′-dideoxy-5′-fluoroadenosine (34 and 35, Respectively). The mixture **32** from the sulfoxide elimination is treated with ethanolic ammonia to give **34** and **35** (an oil), from which **34** is crystallized (EtOAc).

A lengthier procedure to yield **35** is described in the supplementary material, along with additional spectral data.

34: ^{1}H NMR (DMSO-d_6) δ 2.40–2.48 (m, 1 H, H-2′-down), 3.16 (dt, $J \approx$ 6–7 on 13, H-2′-up) 4.98 (m, 1 H, H-3′), 6.65 (d, 1 H, J = 76.5 Hz, vinyl H), 6.70 (t, J = 6.5 Hz, H-1′), 7.35 (s, 2 H, NH_2), 8.17 (s, 1 H, H-3), 8.39 (s, 1 H, H-8).

35: ^{1}H NMR (DMSO-d_6) δ 2.40–2.49 (m, 1 H, H-2′-down), 3.13 (dt, 1 H, J = 6–7.13 Hz, H-2′-up), 5.24 (m, 1 H, H-3′), 5.79 (d, 1 H, J = 5.1 Hz, OH), 6.68 (t, 1 H, J = 7.0 Hz, H-1′), 7.08 (d, 1 H, J = 78.1 Hz, vinyl H), 7.34 (s, 2 H, NH_2), 8.16 (s, 1 H, H-3), 8.37 (s, 1 H, H-8).

Experimental details for compounds **23**, **25**, **27**, **30**, **31**, and **33** are given in the supplementary material.

(*Z*)-9-(5-Deoxy-5-fluoro-β-D-*threo*-pent-4-enofuranosyl)-9*H*-purin-6-amine (36). Nucleoside (*Z*)-**33** (1.070 g, 1.8 mmol) was treated with ethanolic saturated ammonia (room temperature, overnight). The mixture was evaporated and the residue was purified by flash chromatography (EtOAc, then 10% MeOH–90% EtOAc) to afford 344 mg (70%) of solid **36**. Trituration with 5 mL of boiling EtOAc afforded 190 mg (30%) of **36**, mp 203–205 °C dec, after drying at 100 °C in a drying pistol in vacuo. Thermogravimetric analysis revealed that the sample retained EtOAc (7.6% by weight; loss at 100–125 °C): ^{1}H NMR (excluding EtOAc, DMSO-d_6) δ 4.27 (br s, 1 H), 4.63 (br s, 1 H), 5.90 (br s, 1 H, OH), 6.13 (br s, 1 H, OH), 6.61 (d, 1 H, J = 4.1 Hz), 6.67 (d, 1 H, J = 75.9 Hz), 7.34 (br s, 2 H, NH_2), 8.17 (s, 2 H, H-3, H-8) (D_2O exchange sharpened the δ 4.63 signal to a doublet. Irradiation at δ 4.63 (H-3′) produced a NOE on the δ 6.67 (vinyl H) signal as well as the OH signals and H-8.); ^{19}F NMR (DMSO-d_6) −164.89 (d, J = 76.6 Hz); MS (CI/CH_4) m/z 268 $(M + H)^+$; HRMS $(M + H)^+$ calcd for $C_{10}H_{11}FN_5O_3$ 268.0845, found 268.0828. Anal. Calcd ($C_{10}H_{10}FN_5O_3$) C, 44.95; H, 3.77; N, 26.21. Found C, 44.78, H, 4.09; N, 23.50.

(*E*)-9-(5-Deoxy-5-fluoro-β-D-*threo*-pent-4-enofuranosyl)-9*H*-purin-6-amine (37). Nucleoside (*E*)-**33** (414 mg) was treated with ammonia as described for (*Z*)-**33**. The product was crystallized from methanol to afford 99 mg (52%) of **37**: mp 255–260 °C dec; ^{1}H NMR (DMSO-d_6 δ 4.17 (m, 1 H), 4.85 (m, 1 H), 6.05 (d, 1 H, J = 4.9 Hz, OH), 6.15 (d, 1 H, J = 4.4 Hz, OH), 6.60 (d, 1 H, J = 3.5 Hz, H-1′), 7.26 (d, 1 H, J = 79.1 Hz), 8.15 (s, 1 H, H-3), 8.17 (s, 1 H, H-8). (Irradiation at δ 4.85 (H-3′) produced a NOE on the OH-signals and at δ 8.17.); ^{19}F NMR (DMSO-d_6) −181.48 (d, J = 79.2 Hz); HRMS $(M + H)^+$ calcd for $C_{10}H_{11}FN_5O_5$ 268.0845, found 268.0848.

Experimental details for compounds **38**, **39**, **40**, **42**, and **43** are given in the supplementary material. As an example of the sulfuryl chloride reaction, the synthesis of **41** is described below.

N^6,N^6-Dibenzoyl-5′-deoxy-5′-chloro-5′-[(4-methoxyphenyl)sulfinyl]-2′,3′-*O*-isopropylideneadenosine (41). To nucleoside **40** (1.13 g, 1.7 mmol) in 6.5 mL of dry CH_2Cl_2 was added 0.32 mL (4.0 mmol) of pyridine and the mixture cooled in an ice bath under nitrogen. Then 0.18 mL (1.9 mmol) of sulfuryl chloride was added. The mixture was stirred for 20 min and evaporated in vacuo (aspirator, then 1 mm) to afford a foam. The crude product was percolated through a silica gel column (CH_2Cl_2, then 20% EtOAc–80% CH_2Cl_2) followed by a second column to isolate **41** (R_f = 0.22, TLC with 20% EtOAc–CH_cCl_2) as a foam, 0.72 g (60%).

A sample was triturated in CH_2Cl_2–cyclohexane to give a solid, mp 220–228 °C. The remainder of the foam was evaporated from CH_2Cl_2–cyclohexane to give a solid, which was used for the preparation of **42**: ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 3 H), 1.58 (s, 3 H), 3.80 (s, 3 H), 2 m, 1 H, large J = 7.6 Hz), 5.14 (d, 1 H, J = 9.8 Hz), 5.22–5.24 (m, 1 H), 5.60 (d, 1 H, J = 6.3 Hz), 6.57 (s, 1 H), 7.03 (m, 2 H), 7.16 (m, 2 H), 7.46 (m, 4 H), 7.60 (m, 2 H), 7.80 (m, 4 H), 8.716 and 8.722 (2 s, 1 H together), 8.882 and 8.887 (2 s, 1 H, together) (there are at least two isomers whose NMR is almost identical); MS (CI/CH_4) m/z 688 $(M + H)^+$. Anal. ($C_{34}H_{30}ClSN_5O_7$) C, H, N, Cl.

(*Z*)-4′,5′-Didehydro-5′-chloro-5′-deoxyadenosine (44). Nucleoside **43** (98 mg, 0.25 mmol) was treated with saturated methanolic ammonia in a capped tube for 15 h. Solvents were evaporated, and the residue was chromatographed (silica gel, EtOAc, then 10% MeOH–90% EtOAc). Fractions with **44** (R_f = 0.26, 10% MeOH–90% EtOAc) were evaporated and EtOAc was added. Evaporation followed by drying in vacuo at 100 °C afforded 47 mg (65%) of **44**, mp 202–207 °C. Thermogravimetric analysis and NMR indicated that ETOAc (0.05 mol per mol of nucleoside) was retained: ^{1}H NMR (DMSO-d_6) δ 4.73 (t, 1 H, J = 5 Hz), 5.06 (m, 1 H), 5.76 (d, 1 H, J = 7 Hz, OH), 5.81 (d, 1 H, J = 6 Hz, OH), 5.90 (s, 1 H), 6.22 (d, 1 H, J = 8 Hz), 7.36 (br s, 2 H, NH_2), 8.15 (s, 1 H), 8.45 (s, 1 H). (Irradiation at δ 4.73 (H-3′) resulted in a NOE at δ 5.06 (H-2′), 5.81 (3′-OH), and 5.90 (vinyl H)); MS (CI/CH_4) m/z 284 $(M + H)^+$; HRMS (EI) calcd for $C_{10}H_{10}ClN_5O_3$ 283.0472; found 283.0470. Anal. C, 42.34; H, 3.55; N, 24.69; found C, 42.56; H, 3.59; N, 23.83.

Experimental details for compounds **46**, **47**, and **48** are given in the supplementary material.

(*E*)-N^6-Benzoyl-4′,5-didehydro-5′-chloro-5′-fluoro-2′,3′-*O*-isopropylideneadenosine (49). In a 250-mL round-bottom flask a mixture of **48** (2.2 g, 3.12 mmol), 4 mL of diisopropylethylamine, and 80 mL of diglyme was purged with N_2 for 15 min. A condenser was attached and the reaction heated at 145 °C under argon for 18 h. The solvent was removed via Kugelrohr vacuum distillation. The residue was chromatographed on 300 g of silica gel (acetone–$CHCl_3$, 1:3) to afford 622 mg (45%) of **49** as a tan oil: ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3 H), 1.61 (s, 3 H), 5.48 (dd, 1 H, J = 1.2 and 5.9 Hz), 5.84 (d, 1 H, J = 6.0 Hz), 6.37 (s, 1 H), 7.27–8.78 (m, 7 H); ^{19}F NMR ($CDCl_3$) δ −104.03 (s); ^{13}C NMR ($CDCl_3$) 79.47 (d, J_{C-F} = 2.3 Hz); MS (CI/CH_4) m/z 446 $(M + H)^+$.

(*E*)-4′,5′-Didehydro-5′-deoxy-5′-chloro-5′-fluoroadenosine (51). Compound **49** (610 mg, 1.37 mmol) was stirred with 15 mL of 75% trifluoroacetic acid and 25% water for 22 h at ambient temperature. The reaction was concentrated in vacuo and the residue azeotroped twice with EtOH. The residue (**50**) was dissolved in 10 mL of EtOH, cooled to 0 °C and saturated with NH_3. The capped tube was allowed to stand at room temperature overnight. The reaction was concentrated and then purified by

flash chromatography (20% CH_3OH-$CHCl_3$ with 1% NH_4OH) to give 420 mg (100%) **51** as a pale tan oil. The tan oil was dissolved in 15 mL of acetone and placed in a freezer for 4 days. The resulting crystals (170 mg) were collected: mp 232-234 °C; ^1H NMR (CD_3OD) δ 4.78 (d, 1 H, J = 4.2 Hz), 5.14 (dd, 1 H, J = 5.1 and 7.8 Hz), 6.30 (d, 1 H, J = 7.8 Hz), 8.21 (s, 1 H), 8.34 (s, 1 H); ^{19}F NMR (CD_3OD, 282 MHz) δ -110.355 (s); ^{13}C NMR (CD_3OD) δ 74.33 (d, J_{C-F} = 1.95 Hz); MS (CI/CH_4) m/z 302 (M + H)$^+$. Anal. ($C_{10}H_9ClFN_5O_3 \cdot {}^1/_3C_3H_6O$) C, H, N.

(*Z*)-4′,5′-Didehydro-5′-deoxy-5′-chloro-5′-fluoroadenosine (52). Nucleoside **51** in acetone-CH_3OH (2:1) was irradiated for 4 h. The ^{19}F NMR of the reaction indicated a 2:1 mixture of **51** and **52**. Nucleoside **52** was isolated by reverse phase HPLC (Zorbax R_x 250 × 4.6 mm column, mobile phase methanol-water) and characterized by NMR and mass spectra.

The coupling constant of C-3′ to the fluorine was measured for **51** (2.3 Hz) and **52** (0). Comparison to the values recorded for **6** and **13** allowed assignment of olefin geometry: MS (CI/CH_4) m/z 302 (M + H)$^+$; ^1H NMR (CD_3OD) δ 4.80 (d, 1 H, J = 6 Hz), 5.18 (dd, 1 H, J = 5.4 and 7.5 Hz), 6.28 (d, 1 H, J = 7.5 Hz), 8.21 (s, 1 H), 8.34 (s, 1 H); ^{19}F NMR (CD_3OD) δ -126.63 (s).

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Supplementary Material Available: Details of the preparation and assay of rat liver SAH hydrolase and details of the preparation of and spectra for compounds **22-27, 29, 31-35, 38-43, 47,** and **48** (13 pages). Ordering information is given on any current masthead page.

Quinolone Antibacterials: Preparation and Activity of Bridged Bicyclic Analogues of the C_7-Piperazine

John S. Kiely,* Marland P. Hutt, Townley P. Culbertson, Ruth A. Bucsh, Donald F. Worth, Lawrence E. Lesheski, Rocco D. Gogliotti, Josephine C. Sesnie, Marjorie Solomon, and Thomas F. Mich

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.
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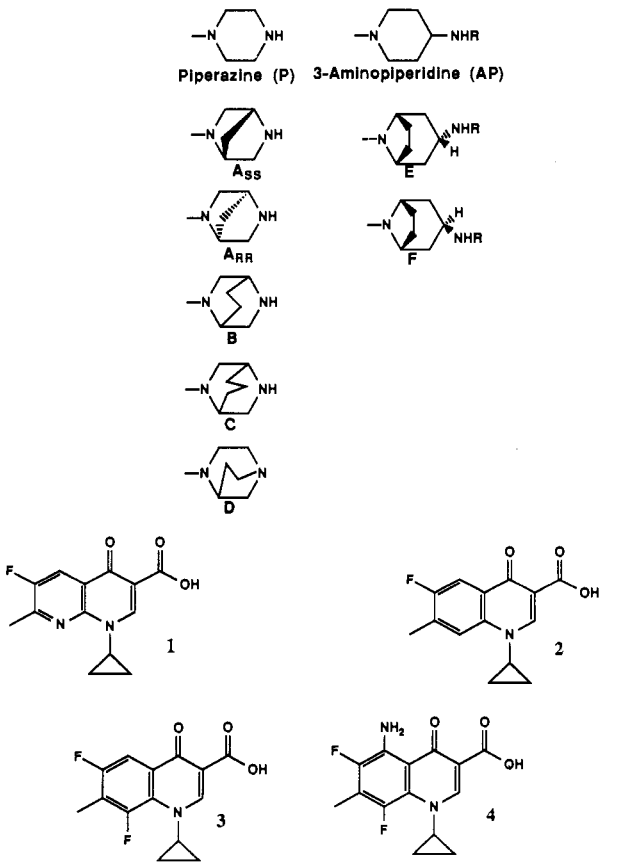
A series of quinolone and naphthyridine antibacterial agents possessing as the C_7-heterocycle bicyclic 2,5-diazabicyclo[*n*.2.*m*]alkanes, where n = 2, 3 and m = 1, 2, and a series including 4-aminopiperidine and 3-amino-8-azabicyclo[3.2.1]octanes have been prepared and evaluated in vitro and in vivo for antibacterial activity against a variety of Gram-negative and Gram-positive organisms. These compounds were also tested against the target enzyme bacterial DNA gyrase. All the examples investigated are nearly equipotent with the parent 7-piperazinyl analogues. Only *endo*-7-(3-amino-8-azabicyclo[3.2.1]oct-8-yl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid displays activity that surpasses that of the piperazine parent.

Introduction

Quinolone antibacterial agents are a major class of antiinfectives with significant potential for continued development.[1] In virtually all modern examples of this antiinfectives class, a nitrogen heterocycle is attached at the C_7-position of the quinolone or naphthyridine nucleus.[2] This moiety plays a significant role in determining antibacterial spectrum and potency and represents a site amenable to significant modification. We report our results from a study replacing the frequently employed 7-piperazinyl substituent with various bicyclic analogues.[3] We were interested in determining the effect these bicyclic C_7-substituents would have on minimum inhibitory concentrations, inhibition of the gyrase enzyme, and in vivo protective doses in mice.

Ciprofloxacin, which contains a 7-piperazinyl moiety, served as the beginning point for our studies. The SAR of the C_7-side chain has been extensively explored[4,5] and the importance of proper side-chain length and ring size has been elucidated.[4] The optimum size for the C_7-substituent has been defined as a ring of five or six atoms.

Chart I



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