10 mM (H_2O) concentrations, respectively, and 298 K temperature. For the D_2O sample the pH was not regulated; however, in H_2O the pH of the original solution was measured to be 8.5. For all the experiments the water peak was used as internal reference (δ = 4.78 ppm). For the D_2O solution no saturation of the residual HDO peak was necessary, while in H_2O the very intense solvent peak was eliminated from the spectrum by using the jump–return–echo sequence.[56] The pH was adjusted by using small amounts of concentrated HCl and NaOH solutions, respectively. Selective irradiation NOE difference spectra were recorded by using low power CW preirradiation of ca. 40 Hz strength for 5 s. Approximate measurement of T_1 have shown values between 0.8 and 2.5 s. An additional relaxation delay was introduced extra to the preirradiation time to avoid saturation resulting in 10-s overall recycling time. Assignments of resonances were straightforward in most cases, except for the protons 2′,2″ and 5′,5″. For the former pair unique assignments were possible according to the NOE effects, while the 5′,5″ signals are still not specifically assigned.

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Supplementary Material Available: Figures of the two major aspects of the H-bond network in NH_2dT and chemical shifts as a function of pH NH_2dT and tables of anisotropic and isotropic displacement coefficients (5 pages); observed and calculated structure factors for 3′-amino-3′-deoxythymidine (8 pages). Ordering information is given on any current masthead page.

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Targeting 5′-Deoxy-5′-(methylthio)adenosine Phosphorylase by 5′-Haloalkyl Analogues of 5′-Deoxy-5′-(methylthio)adenosine

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A series of 5′-haloalkyl-modified analogues of 5′-deoxy-5′-(methylthio)adenosine (MTA), a nucleoside byproduct of polyamine biosynthesis, has been synthesized: 5′-deoxy-5′-[(2-monofluoroethyl)thio]adenosine (**10**), 5′-deoxy-5′-[(2-chloroethyl)thio]adenosine (**4**), 5′-deoxy-5′-[(2-bromoethyl)thio]adenosine (**5**), and 5′-deoxy-5′-[(3-monofluoropropyl)thio]adenosine (**13**). On the basis of their abilities to serve as substrates of MTA phosphorylase prepared from mouse liver, several of these analogues were characterized for their growth inhibitory effects in MTA phosphorylase-containing (murine L5178Y and human MOLT-4) and MTA phosphorylase-deficient (murine L1210 and human CCRF-CEM) leukemia cell lines. The MTA phosphorylase-containing tumor cell lines, especially of human origin, were found to be more sensitive to treatment by these analogues. Of the analogue series, **10** was the most potent inhibitor of growth in each of the cell lines tested. The analogues, especially compound **10**, displayed a reduced capacity to alter polyamine pools relative to MTA, mechanistically indicating a decreased potential for interactions at sites other than MTA phosphorylase. The results indicate that of the analogues tested, compound **10** displayed the best inhibitor/substrate interaction with MTA phosphorylase, which, in turn, correlated with more potent growth inhibition in tumor cell lines containing MTA phosphorylase. Overall, this supports the concept that MTA phosphorylase plays a role in the activation of such analogues.

5′-Deoxy-5′-(methylthio)adenosine (MTA) is a nucleoside metabolite of *S*-adenosylmethionine (AdoMet) produced stoichiometrically during biosynthesis of the polyamines spermidine (SPD) and spermine (SPM). MTA, itself, has well-documented growth inhibitory activity.[1] Although it serves as a substrate of MTA phosphorylase and is known to interact with several other cellular enzyme targets, including SPD synthase, SPM synthase, *S*-adenosylhomocysteine hydrolase, cyclic AMP phosphodiesterase, and adenosine kinase, the exact mechanism of its growth inhibition remains obscure. Numerous analogues of MTA have been synthesized and their chemotherapeutic potential extensively explored.[2,3] Some impetus has come from the observations that certain tumor cell lines[4] and clinically obtained leukemia and solid tumor samples[5,6] have been found to be devoid of MTA phosphorylase activity. However, strategies which successfully exploit this tumor-specific enzyme deficiency have proved elusive.

A critical component of the cellular biochemistry of MTA is MTA phosphorylase, the enzyme which catalyzes the degradation of MTA to adenine and 5-(methylthio)-ribose 1-phosphate (MTRP). Adenine is then recycled via purine salvage pathways, and MTRP is converted via a multistep pathway to methionine. The degradation of MTA by MTA phosphorylase maintains extremely low cellular concentrations of MTA and thereby protects the cell from the growth inhibitory effects of MTA.

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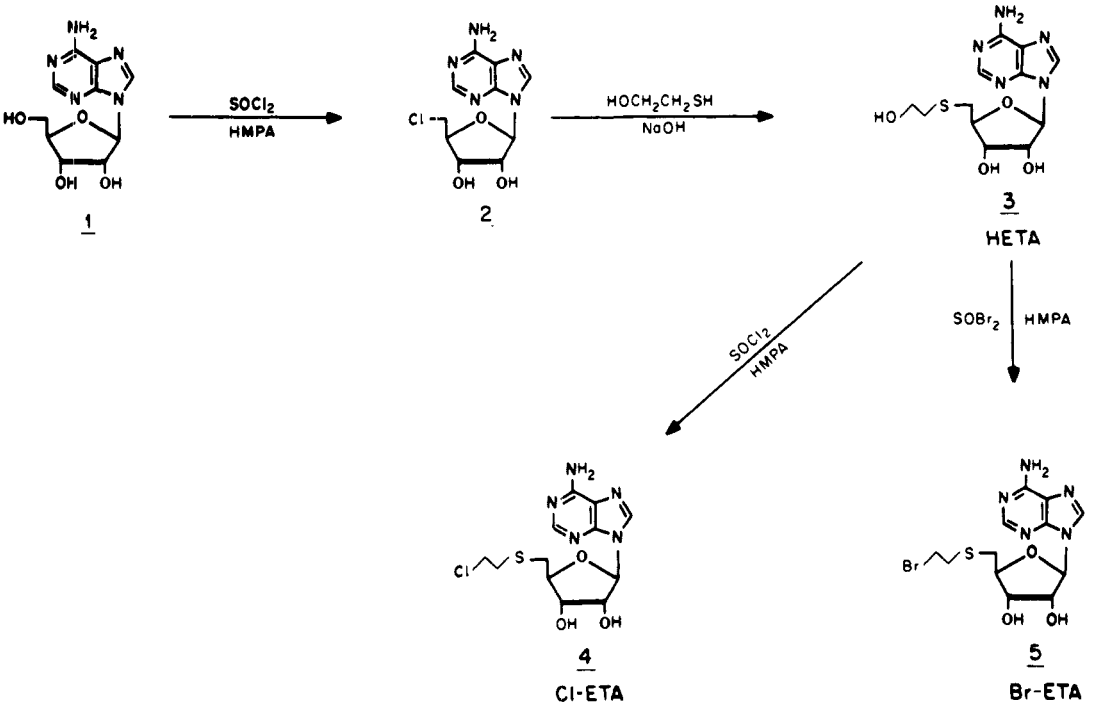
Scheme I



As an approach to the development of MTA analogues as chemotherapeutic agents, Savarese et al.[3] have suggested that MTA analogues which are substrates of MTA phosphorylase might be selectively cytotoxic, based on their ability to produce growth inhibitory metabolites of adenine and/or MTRP; this would interfere with the recycling of purines and/or of methionine in cell types dependent on their salvage.

It was our intent to synthesize analogues of MTA which could be phosphorylyzed by MTA phosphorylase to produce MTRP analogues capable of interfering with methionine recycling, a salvage pathway of potential importance in both tumor cells[3] and pathogenic microorganisms.[7] To test this therapeutic strategy, we initially synthesized 5'-deoxy-5'-[(monofluoromethyl)thio]adenosine (MFMTA) and found this minimally modified analogue of MTA to be a substrate of MTA phosphorylase.[8] However, our expectations that MFMTA would display differential growth inhibitory effects toward L1210 murine leukemia cells which are MTA phosphorylase deficient [MTAPase(-)] and L5178Y murine leukemia cells which contain MTA phosphorylase [MTAPase(+)] were not realized.[8] In a further attempt to obtain analogues which would show increased activity toward MTAPase(+) cell types, we turned to the synthesis of a series of 5'-(haloalkyl) analogues of 5'-deoxy-5'-(ethylthio)adenosine (ETA) and 5'-deoxy-5'-(propylthio)adenosine (PTA), on the basis of the observation that both ETA and PTA are substrates of MTA phosphorylase, with activity comparable to that of MTA itself.[9] These analogues have been evaluated for their biochemical and antiproliferative effects in experimental systems designed to provide information concerning the ability of MTA phosphorylase to serve as an activating or deactivating enzyme for their therapeutic activity.[10] In addition, these analogues have been evaluated to select those that target MTA phosphorylase, while affecting other known sites of MTA action to a lesser degree, or ideally not at all, and that demonstrate potent antiproliferative properties.

Chemistry

The synthetic approach to the described series of 5'-(haloalkyl)-modified analogues of ETA and PTA involves (1) preparation of an alkyl chloride, i.e., 5'-deoxy-5'-chloroadenosine (Scheme I), suitably protected if necessary (Scheme II); (2) reaction of this chloro derivative with an appropriate mercaptoalcohol (i.e. 2-mercaptoethanol or 3-mercaptopropanol) to form the 5'-[(hydroxyalkyl)thio] derivative; (3) reaction of the (hydroxyalkyl)thio derivative with one of the following halogenating agents: for fluorination, diethylamidosulfur trifluoride (DAST); for chlorination, thionyl chloride ($SOCl_2$); for bromination, thionyl bromide ($SOBr_2$); (4) and in the case of the fluorinated derivatives (Scheme II), deprotection to give the desired 5'-[(fluoroalkyl)thio] products. Although the 5'-fluoro, 5'-chloro, and 5'-bromo analogues, **10**, **4**, and **5**, respectively, were obtained in modest yield, this proved to be a consequence of experimental limitations rather than of their inherent chemical instability: in the case of 5'-deoxy-5'-[(2-monofluoroethyl)thio]adenosine (MFETA), the fluorination of 8 with DAST consistently produced a relatively insoluble byproduct whose ^{19}F NMR spectrum

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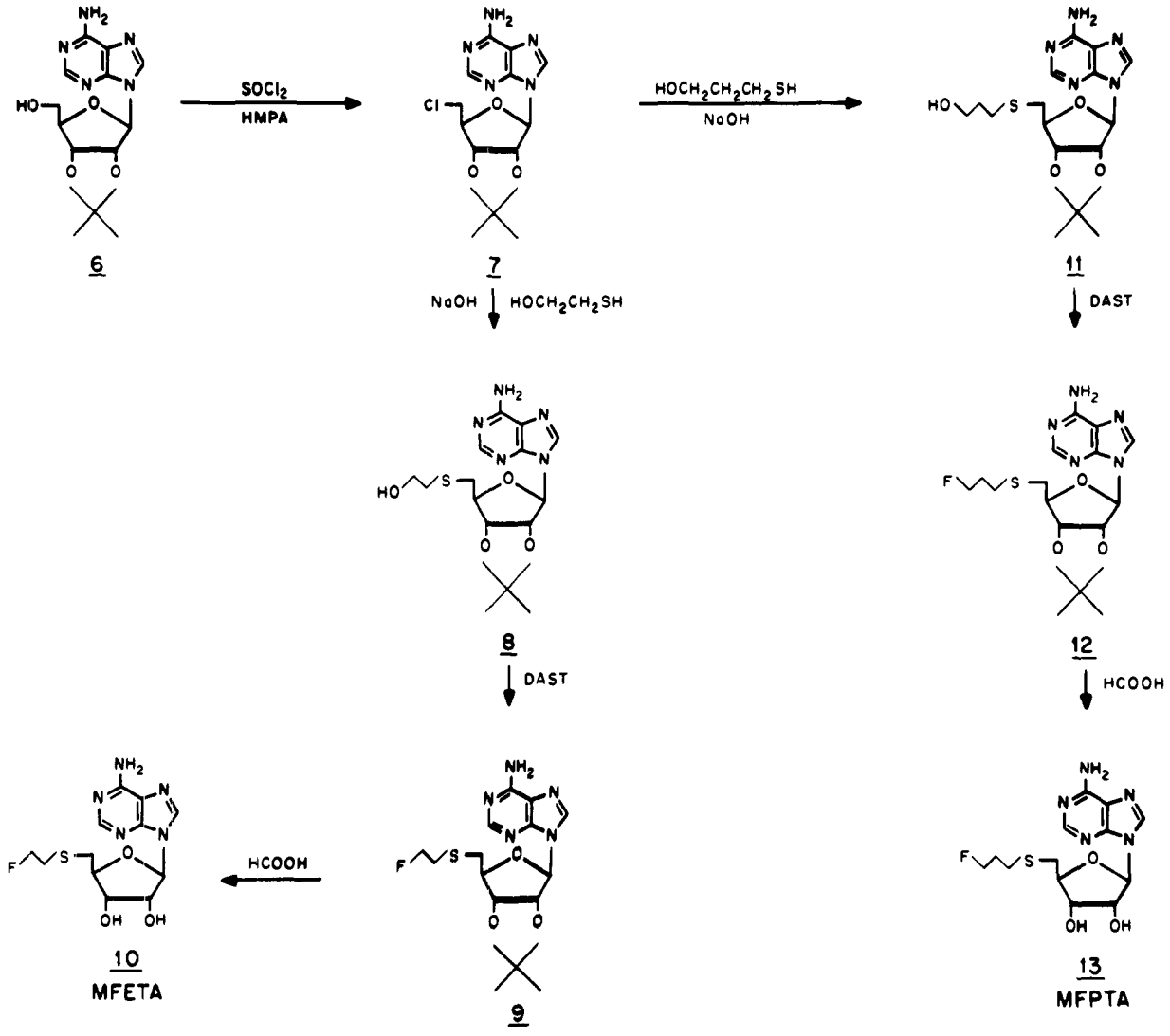
Scheme II



indicated the absence of fluorine and suggested to us the possibility that it may have arisen from competitive dehydration of the starting material; in the instances of Cl-ETA and Br-ETA, removal of hexamethylphosphoramide (HMPA) from crude product mixtures required extraction procedures which removed a significant portion of the product as well. Attempts at further recovery were not made, since additional product could be obtained easily by repeating the halogenation of 5′-deoxy-5′-[(2-hydroxyethyl)thio]adenosine (HETA).

Results and Discussion

Stability of 5′-Haloalkyl Analogues of MTA. The target compounds **4**, **5**, **10**, and **13** are all β-halo thioethers, a class of compounds which are known to undergo facile hydrolysis.[11] Therefore, it was important to determine the stability of these compounds in solution prior to their biochemical characterization: Cl-ETA and Br-ETA, proved to be reasonably stable, undergoing gradual decomposition to HETA when stored in aqueous solution at room temperature for several days; in contrast, solutions of these compounds in ethanol, methanol, or DMSO were completely stable under the same conditions. MFETA proved to be the most stable of the three haloethyl analogues, and various solutions of this compound showed no degradation even after 5 days at 37 °C, conditions which were of relevance for in vivo studies.

Table I. Analogues of ETA and PTA as Substrates and/or Inhibitors of Mouse Liver MTA Phosphorylase

compd	MTA phosphorylase activity	
	K_i, μM	substrate,[a] % control
MTA	1.3 (K_m)	100
ETA	1.9	95
MFETA (10)	3.1	64
Cl-ETA (4)	12	35
Br-ETA (5)	6.5	38
HETA (3)	26	34
PTA	1.7	102
MFPTA (13)	4.0	66

[a] Activity was determined by the change in absorbance at λ 305 following a 10-min incubation when compared to MTA, the control substrate. Values shown are mean of duplicate samples which agreed within 15%.

MTA Phosphorylase Activity. The ability of the newly synthesized MTA analogues to act as substrates for the MTA phosphorylase reaction was determined by using a mouse liver enzyme preparation[8] (Table I). The previously observed efficiency of ETA and PTA as substrates of MTA phosphorylase[9] was confirmed. All of these 5′-(haloalkyl) analogues could serve as substrates, but the two fluorinated analogues, MFETA and MFPTA, were more effectively metabolized (i.e. better substrates) than the 5′-chloro, 5′-bromo, and 5′-hydroxy analogues of ETA.

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Table II. Effects of MTA, ETA, PTA, and 5'-(Fluoroalkyl) Analogues on Growth of Paired (MTA Phosphorylase Containing and MTA Phosphorylase Deficient) Human and Murine Tumor Cell Lines

	IC_{50}, μM[b]			
	mouse (48 h)		human (96 h)	
cell line	L1210	L5178Y	CCRF-CEM	MOLT-4
MTAPase activity[a]	<20	2253	<20	2230
analogue				
MTA	850 ± 120	200 ± 45	150 ± 60	11 ± 7
ETA	800 ± 100	250 ± 100	200 ± 100	20 ± 10
MFETA (10)	150 ± 50	60 ± 35	75 ± 20	10 ± 3
HETA (3)	1000	500	100	40
PTA	1000	500	160	45
MFPTA (13)	600	600	160	25

[a] Intracellular MTAPase activity expressed in pmol/min per mg of protein. [b] These values represent the average of at least three experiments ± standard deviation where indicated; all other values represent the mean of two separate determinations.

Inhibition constants (K_i values) for each analogue were also determined (Table I) and, when compared to the K_m value of MTA, provide an indication of the relative ability of these analogues to compete with MTA for binding to MTA phosphorylase. With the possible exception of HETA, these analogues appear capable of highly effective interaction with the enzyme. Taken together, the K_i value and relative substrate activity of each analogue indicate a more rapid intracellular metabolism of the two fluorinated compounds, MFETA and MFPTA, than of Cl-ETA, Br-ETA, or HETA. Therefore, MFETA and MFPTA, as well as HETA for comparison, were chosen for further evaluation of their cellular effects.

Growth Inhibition. For each analogue, the concentrations required to inhibit cell growth by 50% (IC_{50} values) are shown in Table II for two murine leukemic cell lines, L1210 and L5178Y [MTAPase(−) and MTAPase(+), respectively] and two human leukemic cell lines, CCRF-CEM and MOLT-4 [MTAPase(−) and MTAPase(+), respectively]. ETA, MFETA, HETA, and PTA had lower IC_{50} values in the L5178Y cells than in the L1210 cells. MFETA was the most potent analogue and demonstrated the most significant difference in IC_{50} values between the two murine lines. This differential is consistent with the possibility that in L5178Y cells, MFETA is cleaved by MTA phosphorylase to the growth inhibitory metabolite, 5-[(2-monofluoroethyl)thio]ribose 1-phosphate, whereas in L1210 cells, which cannot degrade the analogue, MFETA itself, is growth inhibitory but to a lesser extent than its metabolite. The relatively high IC_{50} values for MTA, ETA, and PTA in L1210 cells, most likely relate to their high excretion rates, a mechanism which MTAPase(−) tumor cell lines utilize to protect themselves from the potentially damaging effects of MTA accumulation.[12,13]

The analogues were more potent growth inhibitors in the human CCRF-CEM and MOLT-4 cells and displayed a consistent differential between their IC_{50} values in these cell lines. Enhanced sensitivity of the MOLT-4 [MTAPase(+)] cell line to these compounds is consistent with the notion that the phosphorylase plays a role in their metabolic activation. The greater effectiveness of these compounds in human (MOLT-4) cells versus murine (L5178Y) cells, despite comparable levels of MTAPase activity, may reflect the human cells' longer doubling time which necessitates longer exposure (96 h) to these compounds during IC_{50} determinations. It is noteworthy that MFETA was consistently the most potent analogue in all four cell lines tested. Despite comparable IC_{50} values for MFPTA and MFETA in the two human lines, evidence for additional sites of action (i.e. polyamine pool depletion) decreased our interest in the PTA analogues.

Table III. Effects of MTA, ETA, PTA, and 5'-(Fluoroalkyl) Analogues on Polyamine Pools in L1210 and L5178Y Murine Leukemia Cells

treatment[a] (48 h)	L1210, nmol/10^8 cells			L5178Y, nmol/10^8 cells		
	PUT	SPD	SPM	PUT	SPD	SPM
untreated	0.34[b]	1.82	0.65	0.40	2.06	1.53
MTA	1.93	1.22	0.19	1.85	2.79	0.43
ETA	0.77	1.01	0.54	0.69	1.75	1.29
MFETA (10)	0.56	1.95	0.86	0.27	1.55	1.43
HETA (3)	0.75	1.17	0.58	0.66	1.90	1.50
PTA	0.26	0.57	0.41	0.25	1.18	1.00
MFPTA (13)	0.34	0.99	0.44	0.36	1.79	1.07

[a] The respective IC_{50} values at 48 h shown in Table II were the concentrations used for treatment of each cell line. [b] The values represent the mean of three separate experiments with standard deviations of less than 15%.

Table IV. Effects of DHCA, MTA, ETA, PTA, and 5'-(Fluoroalkyl) Analogues on the Activity of *S*-Adenosylhomocysteine Hydrolase

analogue: conc, μM	% enzyme activity remaining					
	DHCA	MFETA	ETA	MFPTA	PTA	MTA
0.01	89	102	99	97	100	101
0.1	37	91	91	85	90	94
1	1	86	87	87	90	86
10	1	57	52	85	67	66
100	0	25	25	45	45	48

Effects on Polyamine Pools. MTA is a potent product inhibitor of polyamine biosynthesis[14] and significantly alters polyamine content when administered at growth inhibitory concentrations (ref 8 and Table III). In either the L1210 or L5178Y cell line, treatment with the IC_{50} concentration of MTA for 48 h caused putrescine (PUT) to accumulate and SPD and/or SPM pools to decrease—a profile consistent with inhibition of SPD and/or SPM synthase. The propyl analogues, PTA and MFPTA decreased SPD and SPM pools in both murine cell lines. With respect to the ethyl analogues, ETA and HETA increased PUT and decreased SPD levels in L1210 cells, but the effects of all three analogues, ETA, MFETA, and HETA were insignificant in L5178Y cells. It is noteworthy that each analogue tested had a reduced capacity to affect polyamine pools when compared to MTA, and that MFETA, the most potent analogue, dissociated the effects on polyamine pools from growth inhibition in both the MTAPase(+) and MTAPase(−) cells.

***S*-Adenosylhomocysteine Hydrolase Activity.** To determine whether inhibition of *S*-adenosylhomocysteine (AdoHcy) hydrolase might, in part, contribute to the cellular effects of these compounds, the inhibitory activity of a selected number of analogues was measured by using an isolated bovine liver enzyme preparation (Table IV). These compounds inhibit enzyme activity by approxi-

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mately 50% at the following concentrations: MTA, 100 μM; ETA, 10 μM; MFETA, 10 μM; PTA, 100 μM; and MFPTA, 100 μM. These inhibitory effects were modest when compared to those elicited by the potent and specific AdoHcy hydrolase inhibitor, 9-(*trans*-2,*trans*-3-dihydroxycyclopent-4-enyl)adenine[15] (DHCA). Nevertheless they may be substantial, especially in MTAPase(-) tumor cells, treated with analogue concentrations required to achieve 50% growth inhibition (Table II).

In Vivo Antitumor Activity. MFETA is an effective substrate of MTA phosphorylase and is the most potent inhibitor of growth in all cell lines tested and as such, was selected for in vivo characterization of its antitumor effects. Comparative studies were carried out in mice bearing either L1210 or L5178Y leukemia. Consistent with its in vitro activity, MFETA displayed greater antitumor effects in the mice bearing L5178Y leukemia: A sharp dose–response was observed and the analogue produced significant increases in life span of 28% ($p = 0.01$) in L1210 leukemic mice and 62% ($p < 0.01$) in L5178Y leukemic mice at its optimal dosage of 200 mg/kg (daily × 5), when given intraperitoneally. Severe toxicity, including some deaths, occurred at the dose level of 300 mg/kg (daily × 5). Different schedules and modes of administration are currently being investigated to improve the therapeutic efficacy of MFETA.

Conclusions

A series of 5′-(haloethyl) and 5′-(halopropyl) analogues of MTA, consisting of MFETA, Cl-ETA, Br-ETA, and MFPTA has been synthesized: the chloro and bromo analogues, Cl-ETA and Br-ETA, have shown reasonable chemical stability under conditions used in their biochemical characterization; the fluoro analogue, MFETA proved to be completely stable even for long term use during in vivo pharmacological evaluation. The biological effects of these derivatives, as well as the 5′-(2-hydroxyethyl) analogue, HETA, have been examined for possible correlations with their relative activity as substrates of MTA phosphorylase. The analogues have shown varying degrees of substrate activity which can be ranked as follows: MFETA = MFPTA > Cl-ETA = Br-ETA = HETA. On the basis of their abilities to serve as substrates of MTA phosphorylase, these analogues were further characterized in MTAPase-containing and MTAPase-deficient leukemia cell lines of human and murine origins: (1) MTA phosphorylase containing tumor cell lines of murine and especially, of human origin were found to be more sensitive to treatment by these analogues. (2) Of the analogue series, MFETA was consistently the most potent inhibitor of growth in each of the cell lines tested. MFPTA and HETA were the least active analogues against the L1210 and L5178Y cell lines and were not considered worthy of further evaluation in murine tumor models. The great difference in IC_{50} values for MFETA and MFPTA in these murine lines, despite equal activity as substrates of MTA phosphorylase, suggested the diminished biological potential of the 5′-(halopropyl) analogues in this series. (3) All of the 5′-(haloalkyl) analogues of MTA tested to date, including MFMTA from an earlier study,[8] showed a reduced capacity, relative to MTA, to alter intracellular polyamine pools in tumor cells. (4) On the basis of the abilities of these 5′-(haloalkyl) analogues to inhibit AdoHcy hydrolase in vitro, this enzyme might be a possible alternate cellular target which contributes to their growth inhibitory effects.

Of the analogues in this series, MFETA best adhered to the criteria used to select a lead compound for further testing in that (1) it was a good substrate of MTA phosphorylase; (2) it was the most potent inhibitor of cell growth and produced the most consistent growth differential in MTAPase(+) and MTAPase(-) cell lines; and (3) it did not have coincident effects on polyamine pools. In this regard, further evaluation of the antitumor activity in MFETA in a panel of human tumor cell lines is in progress.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Melting points were not recorded for compounds **4**, **5**, **10**, **11**, **12**, and **13**. In these instances, the melting point behavior of analytically pure samples showed softening at a low temperature (40–100 °C) which was not accompanied by any discrete melting point range at higher temperatures. ^{19}F and ^{1}H NMR spectra were routinely recorded on a 60-MHz Varian EM-390 spectrometer with tetramethylsilane (TMS) or $CFCl_3$ used as an internal standard. Decoupling was accomplished on a Bruker 200-MHz spectrometer. Elemental analyses were performed by Robertson Laboratory, Inc., Madison, NJ and were within ±0.4% of calculated values unless indicated. Thin-layer chromatography (TLC) was performed on EM aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm). 5′-Chloro-5′-deoxyadenosine[16] (**2**), 5′-deoxy-5′-[(2-hydroxyethyl)-thio]adenosine[17] (**3**), 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylideneadenosine[16] (**7**), 5′-deoxy-5′-(ethylthio)adenosine,[17] and 5′-deoxy-5′-(propylthio)adenosine[17,19] were obtained by literature procedures. The stability of products **4**, **5**, and **10** was determined under conditions described in this section and is elaborated in the Results and Discussion section.

5′-Deoxy-5′-[(2-chloroethyl)thio]adenosine (4). Thionyl chloride (3.7 mL, 4.3 g, 36.2 mmol) was added to 31 mL of hexamethylphosphoramide (HMPA) at 0 °C with stirring, under nitrogen. After 30 min, **3** (4.0 g, 12.2 mmol) in solid form was introduced slowly to prevent clumping, and stirring was continued an additional 2–4 h at 0 °C until TLC (CH_2Cl_2/MeOH; 21:4) indicated disappearance of starting material. The contents of the reaction flask were then poured onto an ice–water mixture (~200 mL), to form a gummy residue, the pH was adjusted to 9 with ammonium hydroxide and the aqueous mixture was extracted 3 times with 200 mL portions of CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to give an oil (1.9 g). A significant amount of the crude product was partitioned into the aqueous layer together with HMPA and was not recovered. A 1.5-g sample of the obtained oil was dissolved in methanol, 2.5 g of silica gel was added, and the methanolic suspension was evaporated to dryness in vacuo. The resultant impregnated silica gel was applied to the top of a silica gel column (3.4 × 34 cm) prepared in CH_2Cl_2. Elution was performed with successive aliquots of CH_2Cl_2 (125 mL), CH_2Cl_2/CH_3OH (98:2) (250 mL), and CH_2Cl_2/CH_3OH (95:5) (290 mL). In this manner, 450 mg (11%) of **4** was obtained from the final fractions as a white solid, providing a 30% recovery from the crude oil. Traces of HMPA (evident in the NMR spectrum) were removed by thor-

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ough washing of the sample with CCl_4: ^1H NMR (DMSO-d_6) δ 8.32 (s, 1 H, purine H), 8.15 (s, 1 H, purine H), 7.23 (s, 2 H, NH_2), 5.87 (d, 1 H, H-1', J = 6 Hz), 5.50 (d, 1 H, OH), 5.30 (s, 1 H, OH), 4.73 (m, 1 H, H-2'), 4.15 (m, 2 H, H-3' and H-4'), 3.68 (t, 2 H, CH_2Cl, J = 6 Hz), 2.85 (m, 4 H, CH_2S and SCH_2-5'). Anal. ($C_{12}H_{16}N_5O_3ClS$) C, H, N, S. The stability of 4 in solutions of water, methanol, or DMSO at room temperature for 5 days was determined by using TLC and HPLC conditions described for compound 10.

5'-Deoxy-5'-[(2-bromoethyl)thio]adenosine (5). Thionyl bromide (1.4 mL, 3.8 g, 18.3 mmol) was added to 9.5 mL of HMPA cooled at 0 °C under argon. The solution was allowed to come to room temperature over 30 min and **3** (4.0 g, 12.2 mmol) was added in portions as a dry solid. TLC (CH_2Cl_2/MeOH; 21:4) showed the reaction to be complete by the end of 3 h at which time a 400 mL mixture of ice and water was added. The reaction mixture was stirred until complete dissolution was achieved, and the pH was adjusted within the range of 9–10 at 0 °C with ammonium hydroxide. The cold aqueous mixture was extracted 3 times with 150-mL portions of ethyl acetate. The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated in vacuo to give a semisolid residue. Coevaporation with CH_2Cl_2 gave a white solid residue which was thoroughly washed with CCl_4 to remove excess HMPA. The residue was applied to a silica gel column (3.4 × 34 cm) and eluted as described above for **4**. The product, which was the last compound to pass through the column, was separated from a second, closely running, upper component. In this manner, 200 mg (4.2%) of **5** was obtained as a white solid: ^1H NMR (DMSO-d_6) δ 8.40 (s, 1 H, purine H), 8.20 (s, 1 H, purine H), 7.48 (s, 2 H, NH_2), 5.91 (d, 1 H, H-1', J = 6 Hz), 4.60 (m, 1 H, H-2'), 4.08 (m, 2 H, H-3' and H-4'), 3.50 (m, 4 H, CH_2Br and CH_2S) and 2.85 (m, 2 H, SCH_2-5'). Anal. ($C_{12}H_{10}N_5O_3SBr$) C, H, N, S. The stability of **5** in solutions of water, methanol, or DMSO at room temperature for 5 days was determined by using TLC and HPLC conditions described for compound 10.

5'-Deoxy-5'-[(2-hydroxyethyl)thio]-2',3'-O-isopropylideneadenosine (8). A solution of 2-mercaptoethanol (21.7 mL, 24.2 g, 309 mmol) and sodium hydroxide (12.65 g, 316 mmol) in 72 mL of water was stirred with cooling for 30 min. The solution was then transferred to a 500-mL beaker and 5'-chloro-5'-deoxy-2',3'-*O*-isopropylideneadenosine[16] (**7**) (42 g, 129 mmol) was added with stirring. The resultant mixture was slowly heated to 105–108 °C and its volume increased to approximately 200 mL by addition of water. Heating and stirring were continued an additional 1.5–2 h at which time TLC (silica gel, CH_2Cl_2/MeOH; 22:3) indicated disappearance of starting material and volume was decreased by one half. The reaction mixture was cooled in an ice bath, the aqueous layer decanted, and the highly viscous organic layer dissolved in approximately 200 mL of CH_2Cl_2. This was washed with 3 × 75 mL of H_2O, dried over $MgSO_4$, and filtered. The filtrate was stored below 0 °C for several days, allowing the product to crystallize. Recrystallization from CH_2Cl_2/MeOH/petroleum ether yielded a first crop of 18.4 g and upon further cooling of the mother liquor, a second crop of 4.9 g. Total recovered yield of **8** was 22.5 g (47.5%): mp 136–137 °C; ^1H NMR (Me_2SO-d_6) δ 8.35 (s, 1 H, purine), 8.20 (s, 1 H, purine), 7.35 (s, 2 H, NH_2), 6.20 (d, 1 H, H-1', J = 6 Hz), 5.50 (dd, 1 H, H-2'), 5.0 (dd, 1 H, H-3'), 4.75 (t, 1 H, OH), 4.25 (m, 1 H, H-4'), 3.5 (q, 2 H, CH_2OH) 2.80 (d, 2 H, CH_2S), 2.60 (t, 2 H, SCH_2-5'), 1.55 (s, 3 H, CH_3-C), 1.35 (s, 3 H CH_3-C). Anal. ($C_{15}H_{21}N_5O_4S$) C, H, N, S.

5'-Deoxy-5'-[(2-monofluoroethyl)thio]-2',3'-O-isopropylideneadenosine (9). A dried 500-mL round bottom flask containing **8** (10.0 g, 27.2 mmol) and a magnetic stirring bar was charged with argon and capped with a rubber septum. Dry CH_2Cl_2 (250 mL) was injected and the flask then cooled with stirring in a dry ice/ethanol bath for 20 min. To this was slowly injected diethylamidosulfur trifluoride (DAST) (10.8 mL, 13.2 g, 81.7 mmol). After 40 min the flask was transferred to an ice/water bath and after 1.5–2 h, when TLC (CH_2Cl_2/MeOH; 22:3) indicated disappearance of starting material, the reaction mixture was poured into 350 mL of ice cold saturated aqueous sodium bicarbonate. A yellow copious precipitate formed immediately, which remained associated with the aqueous layer, after its extraction with 3 × 300 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and evaporated in vacuo to give a 2.2 g of a residual foam. This was applied to a Florisil column in CH_2Cl_2 and chromatographed with 500 mL of CH_2Cl_2 and then CH_2Cl_2 containing 1% MeOH. In this manner, 1.28 g (12.7%) of **9** was obtained as a white solid. Recrystallization from EtOAc/Et_2O/hexane gave an analytically pure sample of **9**: mp 113–114 °C; ^1H NMR ($CDCl_3$) δ 8.30 (s, 1 H, purine), 7.90 (s, 1 H, purine), 6.73 (s, 2 H, NH_2), 6.10 (d, 1 H, H-1', J = 2.5 Hz), 5.50 (dd, 1 H, H-2', J = 2.5 and 6 Hz), 5.05 (dd, 1 H, H-3', J = 3 and 6 Hz), 4.50 (dt, 2 H, FCH_2, J = 6 and 46.5 Hz), 4.40 (m, 1 H, H-4'), 3.0–2.6 (m, 4 H, CH_2S and SCH_2-5'), 1.60 (s, 3 H, CH_3C), 1.40 (s, 3 H, CH_3C); ^{19}F NMR ($CDCl_3$) δ −208.4 (m). Anal. ($C_{15}H_{20}N_5O_3SF$) C, H, N, S.

5'-Deoxy-5'-[(2-monofluoroethyl)thio]adenosine (10). Compound **9** (2.4 g, 3.5 mmol) was dissolved in 43 mL of 70% formic acid and stirred overnight at room temperature. Solvent was then removed in vacuo to give a multicomponent, oily residue, as determined by TLC (CH_2Cl_2/MeOH; 22:3). The residue was repeatedly coevaporated with water and then methanol to remove traces of formic acid. The residue was then dissolved in a minimum amount of methanol and applied to a silica column as a 10% MeOH/CH_2Cl_2 solution. Further elution and fractionation gave **10** (1.26 g, 59%) as a white solid: ^1H NMR (DMSO-d_6) δ 8.30 (s, 1 H, purine), 8.13 (s, 1 H, purine), 7.20 (s, 2 H, NH_2), 5.83 (d, 1 H, H-1', J = 6 Hz), 5.40 (s, 1 H, OH), 5.21 (s, 1 H, OH), 4.8–4.55 (m, 2 H), and 4.22–3.8 [(2m, 3 H, (note: these two multiplets contain H-2', H-3', H-4', and CH_2F). The characteristic doublet of triplets for CH_2F can be discerned among these two multiplets, with each containing one triplet, J = 46 Hz)], 3.0–2.55 (m, 4 H, CH_2S and SCH_2, H-5'); ^{19}F NMR (DMSO-d_6) δ −206.4 to −208 (tt, centered at −207.2, J = 46 and 21 Hz). An analytically pure sample was obtained by recrystallization from methanol. Anal. ($C_{13}H_{16}N_5O_3SF$·2/3CH_3OH) C, H, N.

The stability of MFETA in solution, under the following conditions was determined by TLC (CH_2Cl_2/MeOH; 22:3) or HPLC: (1) EtOH or MeOH, room temperature, 5 days or (2) Molecusol or 50% aqueous DMSO, 37 °C, 5 days. HPLC was carried out by using a Beckman 110 pump with a reverse-phase column. Under conditions of isocratic elution with a 2.5 mM phosphate buffer at pH 6, containing 20% MeOH, MFETA had a retention time of approximately 24 min, and HETA, approximately 11 min.

5'-Deoxy-5'-[(3-hydroxypropyl)thio]-2',3'-O-isopropylideneadenosine (11). By using a procedure similar to that described above for synthesis of **8**, a reaction mixture initially containing **7** (10.0 g, 30.7 mmol), 3-mercapto-1-propanol (6.8 g, 6.5 mL, 73.7 mmol) and NaOH (3.02 g, 75.4 mmol) in H_2O, was heated at 93 °C for 2 h, worked up, and chromatographed on silica gel to give **11** (8.9 g, 76.3%) as a white solid: ^1H NMR ($CDCl_3$) δ 8.30 (s, 1 H, purine), 7.93 (s, 1 H, purine), 6.33 (s, 2 H, NH_2), 6.03 (d, 1 H, H-1', J = 2 Hz), 6.47 (dd, 1 H, H-2', J = 2 and 6 Hz), 5.0 (dd, 1 H, H-3', J = 3 and 6 Hz), 4.36 (m, 1 H, H-4'), 3.64 (t, 2 H, CH_2OH), 2.87–2.46 (m, 4 H, CH_2S and SCH_2-5'), 1.70 (quintet, 2 H, CH_2), 1.59 (s, 3 H, CH_3C), 1.36 (s, 3 H, CH_3C). Anal. ($C_{16}H_{23}N_5O_4S$) C, H, N, S.

5'-Deoxy-5'-[(3-monofluoropropyl)thio]adenosine (13). By using the same procedure described above for preparation of **9**, a solution of **11** (2.5 g, 6.6 mmol) in 70 mL of dry CH_2Cl_2, immersed in a dry ice/ethanol bath, was slowly injected with DAST (3.17 g, 2.6 mL, 19.2 mmol) and then transferred to an ice/water bath after 40 min. An additional 1.5 mL of DAST was added at 90 min and the reaction worked up at the end of 2 h. Chromatography on Florisil afforded **12** (400 mg, 16%) as a white solid: ^1H NMR ($CDCl_3$) δ 8.20 (s, 1 H, purine), 7.86 (s, 1 H, purine), 6.43 (s, 2 H, NH_2), 6.0 (d, 1 H, H-1', J = 2.5 Hz), 5.47 (dd, 1 H, H-2', J = 2.5 and 6 Hz), 5.02 (dd, 1 H, H-3', J = 3 and 6 Hz), 4.65 and 4.03 (2t, 2 H, CH_2F, J = 4.5 Hz and 49 Hz), 4.34 (m, 1 H, H-4'), 2.90–2.46 (m, 4 H, CH_2S and SCH_2-5'), 2.14–1.62 (m, 2 H, CH_2), 1.60 (s, 3 H, CH_3C) and 1.40 (s, 3 H, CH_3C); ^{19}F NMR ($CDCl_3$) −217 (tt, J = 27 and 49 Hz). Compound **12** was then stirred overnight in 6 mL of 70% formic acid. After removal of the solvent in vacuo, the residue was applied to two preparative TLC plates (20 × 20 cm, 1000 μm) and the plates developed in a chamber with CH_2Cl_2 (210 mL)/MeOH (40 mL). The band containing product was removed, extracted with 30% methanolic CH_2Cl_2, and evaporated to afford **13** (242 mg, 70.5%): ^1H NMR (DMSO-d_6) δ 8.32 (s, 1 H, purine), 8.18 (s, 1 H, purine), 7.25 (s,

2 H, NH_2), 5.86 (d, 1 H, H-1'), 5.47 (d, 1 H, OH, J = 6 Hz), 5.30 (d, 1 H, OH, J = 4.5 Hz), 4.90–4.50 and 4.30–3.80 (2 m, 5 H, H-2', H-3', H-4', and CH_2F), 3.0–2.3 (2m, 4 H, CH_2S and SCH_2-5') and 1.8 (m, 2 H, CH_2); ^{19}F NMR (DMSO-d_6) -214.9 (tt, J = 25.5 and 49.5 Hz). A recrystallized analytical sample was obtained by dissolving the sample in methanol (1 vol) and adding successive volumes of CH_2Cl_2 (1/2), Et_2O (1/4), and petroleum ether (1/3). Anal. Calcd for ($C_{13}H_{16}N_5O_3SF \cdot 2/3CH_3OH$): C, 45.00; H, 5.71; N, 19.20. Found: C, 44.52; H, 5.27; N, 19.11.

Biological Methods. MTA Phosphorylase Activity. Enzyme activity was determined by measuring the conversion of [*methyl*-^{3}H]MTA to [*methyl*-^{3}H]MTRP in a mouse liver preparation as previously described.[8] Kinetic constants for each analogue were determined by Lineweaver–Burk plots. The ability of each analogue to act as a substrate for MTA phosphorylase was determined by the spectrophotometric assay of Savarase et al.[20]

Cell Growth. L1210, L5178Y, and MOLT-4 cell lines were grown in RPMI 1640 and 10% Nu Serum (Collaborative Research Inc., Lexington, MA) and CCRF-CEM cells were grown in 10% Horse Serum (Gibco Laboratories, Grand Island, NY) and maintained as previously described for suspension cultures.[21] Cell cultures (0.3×10^5 cells/mL) were treated with each compound at 0.1–1000 μM to determine the concentration that inhibited growth by 50% (IC_{50}) at 48 h for the L1210 and L5178Y cells and at 96 h for the CCRF-CEM and MOLT-4 cells. The incubation times allowed approximately 4 cell doublings in each cell line. All compounds were freshly prepared prior to treatment by dissolving in DMSO and diluting in serum-free media. Cells were counted by electron particle counting (Model XF Coulter Counter; Coulter Electronics, Hialeah, FL).

Polyamine Pools. Following treatment with each compound at the IC_{50} concentration for 48 h in L1210 and L5178Y cells or for 96 h in CCRF-CEM and MOLT-4 cells, a portion of the cells was washed with phosphate-buffered saline and extracted with 0.6 N perchloric acid for polyamine pool determinations by HPLC techniques previously described.[22]

***S*-Adenosylhomocysteine Hydrolase Activity.** Enzyme activity was determined by using a bovine liver preparation as previously described.[15]

In Vivo Therapeutic Evaluation of MFETA. The antitumor effects of MFETA were evaluated in DBA/2N mice (Charles River) who received intraperitoneal transplants of 10^6 L1210 leukemic cells or 10^6 L5178Y leukemia cells on day 0 according to protocols described previously.[23] In two separate experiments, groups of mice (5 to 10 per group) were treated with dosages of 10, 50, 100, 200, or 300 mg/kg MFETA, intraperitoneally on days 1, 2, 3, 4, and 5, following tumor implantation. MFETA was formulated for administration in Molecusol. The percent increase in life span (% ILS) of treated animals relative to untreated animals was used as the measure of their therapeutic effectiveness at specified dose regimens.

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