the test compound on contractile force (CF) were compared to pretreatment control values and expressed as a percent change.

Renal Vasodilating Activity.[16] Adult mongrel dogs were anesthetized (sodium pentobarbital; 45 mg/kg ip) and surgically prepared for electromagnetic measurement of renal artery blood flow. A femoral artery was cannulated for measuring arterial blood pressure, and drugs were administered intravenously via the femoral vein. Heart rate was monitored with a cardiotachometer. Renal vascular resistance was calculated as the ratio of mean arterial blood pressure to renal artery blood flow. Dopamine was infused intravenously at 3 μg/kg per min for 10 min (at an infusion rate of about 1 mL/min) to determine responsiveness of each dog to a known renal vasodilator. At this dose, dopamine increases renal blood flow approximately 30% without appreciable α or β adrenergic receptor mediated vasoconstriction and cardiac stimulation. Cumulative dose–response data were obtained in groups of two or more dogs by administering the test compound at progressively increasing infusion rates (normally 5-fold increments: 0.2 and 1.0 mg/kg for the cumulative 1.2 mg/kg dose, and 0.2, 1.0, and 5.0 mg/kg for the cumulative 6.2 mg/kg dose), each dose being infused for 5 min. Compounds were solubilized in 0.9% physiological saline with some requiring aqueous acid or base (0.1–1.0 mequiv) for solubilization. These vehicles alone were shown not to alter any measured parameter by more than ±5% of baseline values. All animals were monitored for at least 30 min postdrug treatment and the maximum percent change from predrug control was quantitated for renal artery blood flow (RBF), renal vascular resistance (RVR), mean arterial blood pressure (MAP), and heart rate (HR). At the infusion dose of 3 μg/kg per min under the conditions described above, dopamine provided the following percent changes in the measured parameters (n = 25): RBF = +32 ± 3; RVR = −27 ± 2; MAP = −6 ± 1; HR = +5 ± 2.

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Acknowledgment. We thank Dr. M. L. Cotter and her staff for microanalytical data and spectral data. We also are grateful to Robert Mallory for the preparation of bromoindanone **10b**.

Registry No. **10b**, 18028-29-0; **11b**, 118575-95-4; **12b**, 118575-96-5; **12c**, 118575-92-1; **13b**, 114130-58-4; **15** (R = 7-OMe; n = 1), 118575-97-6; **15** (R = 6-OMe, 7-OMe, 8-OMe; n = 3), 118575-99-8; **15** (R = 7-OMe, 8-OMe; n = 2), 118576-00-4; **16** (R = 6-OH, 7-OH; n = 2; R_4 = Bu), 118575-98-7; **19**, 113982-73-3; **20**, 113982-75-5; **21**, 113982-76-6; **22**, 113982-79-9; **23**, 113982-88-0; **24**, 113983-47-4; **25**, 118576-01-5; **26**, 16535-98-1; **27**, 114130-71-1; **28**, 113983-32-7; **28** diacetate, 118575-90-9; **29**, 113982-78-8; **30**, 113982-77-7; **31**, 113982-86-8; **32**, 113982-85-7; **33**, 118576-02-6; **34**, 113982-84-6; **34** acetate, 118575-91-0; **35**, 113983-40-7; **36**, 113983-45-2; **37**, 118576-03-7; **38**, 113983-08-7; **39**, 113982-89-1; **40**, 113983-46-3; **41**, 113983-13-4; **42**, 113983-23-6; **43**, 118576-04-8; **44**, 113983-29-2; **45**, 118576-05-9; **46**, 113983-35-0; **47**, 114052-45-8; **48**, 113982-87-9; **49**, 113983-02-1; **50**, 113983-00-9; **51**, 113983-11-2; **52**, 113983-21-4; **53**, 113983-42-9; **54**, 113982-91-5; **55**, 118597-18-5; **56**, 118576-06-0; **57**, 118576-07-1; **58**, 113983-44-1; **59**, 113983-31-6; 3-hydroxy-6,7-dimethoxy-1-methylisoquinoline, 16535-98-1; 3-hydroxy-7,8-dimethoxy-1-methylisoquinoline, 114130-58-4; n-butyl chloroformate, 592-34-7; 2-acetyl-6-bromo-3,4-dimethoxyphenylacetic acid, 118575-93-2; 4-amino-7-ethoxy-3-hydroxy-6-methoxy-1-methylisoquinoline, 118575-94-3; allyl chloroformate, 2937-50-0.

Synthesis and Antiproliferative Effects of Novel 5′-Fluorinated Analogues of 5′-Deoxy-5′-(methylthio)adenosine

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5′-Deoxy-5′-[(monofluoromethyl)thio]adenosine (**9**) and 5′-deoxy-5′-fluoro-5′-(methylthio)adenosine (**10**), two novel analogues of 5′-deoxy-5′-(methylthio)adenosine (MTA), have been synthesized and evaluated for their substrate and inhibitory activities toward MTA phosphorylase and for their biological effects in L1210 (MTA phosphorylase deficient) and L5178Y (MTA phosphorylase containing) murine leukemia cell lines. Compound **9** was a potent competitive inhibitor of MTA phosphorylase with a K_i value of 3.3 μM and was also a substrate, with activity approximately 53% that of MTA. Compound **10** was significantly less inhibitory toward the phosphorylase with a K_i value of 141 μM; its lack of substrate activity was attributed to rapid nonenzymatic degradation. The 50% growth inhibitory concentrations (48 h) of **9** were 300 and 200 μM in L1210 and L5178Y cells, respectively; for **10**, these respective values were 2 and 0.7 μM. The initial characterization of **9** in these systems reveals that it differs from MTA by not acting as a product regulator of the polyamine biosynthetic pathway.

5′-Deoxy-5′-(methylthio)adenosine (MTA) (**3**) is a metabolite of *S*-adenosylmethionine (AdoMet), which is formed as a byproduct during synthesis of the polyamines, spermidine (Spd), and spermine (Spm). The maintenance of low intracellular levels of MTA via further metabolism is of critical importance, since the nucleoside is a relatively potent inhibitor of cell growth. The enzyme MTA phosphorylase is responsible for the degradation of MTA to adenine and 5-(methylthio)ribose 1-phosphate (MTRP). Adenine is subsequently salvaged to the nucleotide pools, and MTRP is recycled to methionine by a multistep pathway which has not been fully elucidated.[1] Interest in chemotherapeutic strategies involving MTA and its analogues has been stimulated by Toohey's discovery in 1977 that certain malignant cell lines are devoid of MTA phosphorylase activity[2] and by the more recent documentation of MTA phosphorylase deficiency in a significant portion of clinically obtained human leukemias and solid tumors.[3,4]

Numerous analogues of MTA have been synthesized[5,6] and characterized in tumor cell lines containing or lacking

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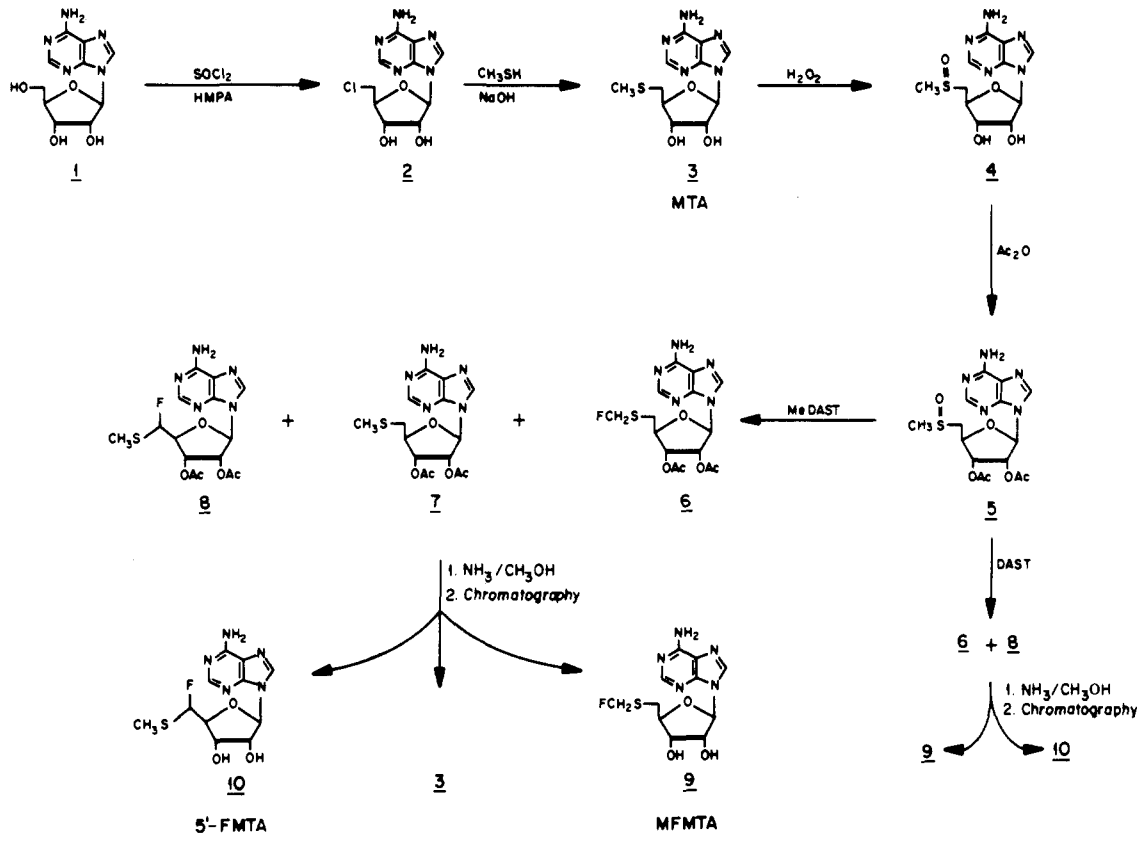
Scheme I



MTA phosphorylase [MTAPase(+) or MTAPase(−)].[6,7] Analogues that serve as substrates for the phosphorylase can be degraded intracellularly, giving analogues of MTRP and adenine that may, themselves, be potentially toxic,[8] whereas these same MTA analogues remain structurally intact in tumor cells lacking the phosphorylase. Thus, MTA analogues can be appropriately designed to exert growth-inhibitory effects by two distinctly different mechanisms, depending on the presence or absence of MTA phosphorylase in a given tumor cell population. Among the analogues potentially capable of this dual mode of action are 5′-deoxy-5′-[(monofluoromethyl)thio]-adenosine (MFMTA) (**9**) and 5′-deoxy-5′-fluoro-5′-(methylthio)adenosine (5′-FMTA) (**10**). Cells lacking the phosphorylase might be highly susceptible to direct growth-inhibitory effects caused by these closely related structural analogues of MTA while cells containing the phosphorylase might metabolize MFMTA or 5′-FMTA to 5-[(monofluoromethyl)thio]ribose 1-phosphate or 5-fluoro-5-(methylthio)ribose 1-phosphate, two potentially toxic metabolites capable of interfering with recycling of MTRP to methionine.

Synthesis of MFMTA and 5′-FMTA and evaluation of their substrate and inhibitory activities toward MTA phosphorylase and their antiproliferative effects in cultured L1210 leukemia cells [MTAPase(−)] and cultured L5178Y leukemia cells [MTAPase(+)] are described in this report.[9]

Chemistry

A key aspect of the synthetic route developed for MFMTA and 5′-FMTA is the utilization of a general procedure for transformation of sulfoxides to α-fluoro thioethers by (diethylamino)sulfur trifluoride (DAST), reported by McCarthy et al.[10] As outlined in Scheme I, this involves a three-step conversion of adenosine (**1**) to MTA sulfoxide (**4**) by established literature procedures.[11,12] The choice of protecting groups for the *cis*-diol functionality of **4** was directed by the need to avoid acidic conditions for its removal, since α-fluoro thioethers are reported to be highly unstable under these circumstances.[13] Accordingly, **4** was protected as its 2′,3′-di-*O*-acetate (**5**). When **5** was reacted with DAST, although the desired product **6** was formed, compound **8** was the predominant product. The formation of **8** was unexpected, on the basis of the sterically controlled, regioselective mechanism for formation of α-fluoromethyl sulfides from methyl sulfoxides proposed by McCarthy et al.[10]

In an attempt to increase the yield of **6**, (dimethylamino)sulfur trifluoride (meDAST) was used in place of DAST. Although the reaction with meDAST was cleaner and gave slightly higher overall yields of **9** and **10**, the relative amounts of the two compounds formed remained essentially unchanged; after deprotection of **6** and **8** in ammmonia/methanol, small amounts of a third product, MTA (**3**), were isolated in addition to **9** and **10**. This suggested that 2′,3′-di-*O*-acetyl-MTA (**7**) was also formed in the meDAST reaction. This was confirmed by preparing

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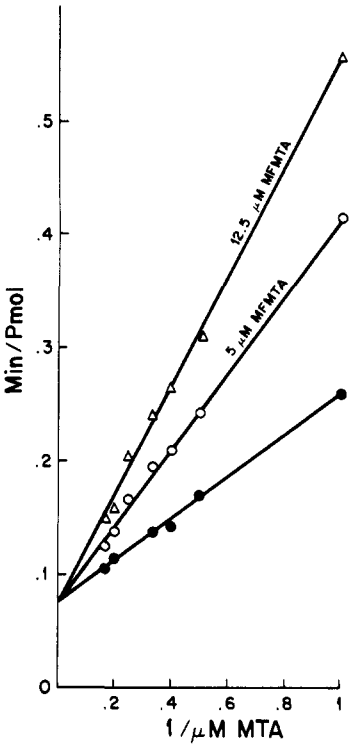

Figure 1. Inhibition of MTA phosphorylase by MFMTA. (●) Control.

an authentic sample of **7** for spectral comparisons. The methods developed for purification and separation of **3**, **9**, and **10**, are noteworthy. Conventional column chromatography on silica, alumina, or Florisil caused apparent degradation of the α-fluoro thio components **9** and **10**. However, use of Bio-Rad AG1-X8 (OH^-) anion-exchange resin not only removed ammonium acetate (formed as a byproduct in the ammonia/methanol deprotection reaction) but also facilitated chromatographic resolution of the closely eluting compounds **3**, **9**, and **10**. Apparently, under the described circumstances, the anion-exchange resin acted as an affinity matrix for these three nucleosides. Further separation of the diastereomeric components of **10** was not attempted.

Results and Discussion

MTA Phosphorylase. Mouse liver MTA phosphorylase showed a K_m for MTA of 1.4 ± 0.2 μM. Both MFMTA and 5'-FMTA were competitive inhibitors of MTA as shown by Lineweaver–Burk plots (Figures 1 and 2). The K_i values of MFMTA and 5'-FMTA were 3.3 ± 0.6 μM and 141 ± 5 μM, respectively. MFMTA was not only a more potent inhibitor compared to 5'-FMTA but was also a substrate for the enzyme with activity approximately 53% that of MTA. 5'-FMTA showed immediate breakdown, apparently to adenine and unknown byproducts, upon dilution in water. Since this rapid degradation was not dependent upon the presence of MTA phosphorylase, the inherent instability of 5'-FMTA in aqueous medium became apparent. It is of interest to note that an analogue closely related to MFMTA and 5'-FMTA, 5'-deoxy-5'-[(difluoromethyl)thio]adenosine (DFMTA), was also found to be a potent inhibitor of MTA phosphorylase but was not a substrate of the enzyme.[14]

Growth Inhibition. The concentrations of MTA and its analogues MFMTA, 5'-FMTA, and 5'-deoxy-5'-iodo-

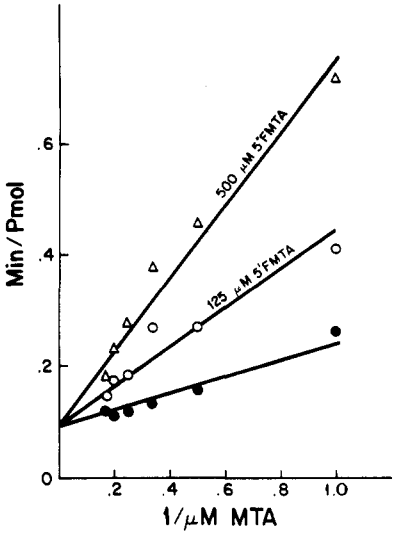

Figure 2. Inhibition of MTA phosphorylase by 5'-FMTA. (●) Control.

Table I. Effects of MTA and Its Analogues on the Growth of L1210 and L5178Y Murine Leukemia Cells

	IC_{50}, μM	
treatment (48 h)	L1210	L5178Y
MTA (**3**)	600	60
5'-FMTA (**10**)	2	0.7
MFMTA (**9**)	300	200
5'-IAdo	300	2

adenosine (5'-IAdo) needed to inhibit growth by 50% at 48 h (IC_{50} values) were determined in L1210 cells [MTAPase(−)] and L5178Y cells [MTAPase(+)] (Table I). 5'-IAdo, a substrate of MTA phosphorylase known to be more toxic to phosphorylase-containing (L5178Y) cells than to phosphorylase-deficient (L1210) cells,[15] was included in this study for comparative purposes. In general, L5178Y cells were more sensitive than L1210 cells to all of the compounds. This effect was more pronounced for MTA and 5'-IAdo, suggesting that the presence of MTA phosphorylase contributed to their toxicity in L5178Y cells. Correspondingly, the lack of differential sensitivity between L1210 and L5178Y cells to MFMTA suggests that its metabolic degradation by the phosphorylase in L5178Y cells did not produce any metabolites with significantly greater toxicity than MFMTA itself. Although MFMTA was found to be an effective inhibitor as well as a substrate of the phosphorylase, the cellular mechanisms by which it causes growth inhibition do not appear related to this enzyme. Despite its rapid nonenzymatic breakdown, 5'-FMTA was significantly growth inhibitory in both cell lines. These effects, which were highly reproducible, can most likely be attributed to degradation products of the carbohydrate portion of 5'-FMTA, since adenine is nontoxic at concentrations as high as 100 μM.[16]

Effects on Polyamine Biosynthesis. MTA is a known product inhibitor of Spm synthase and, to a lesser extent, of Spd synthase.[17] Accordingly, MFMTA was compared to MTA for its effects on polyamine pools (Table II). At its IC_{50} concentrations, MFMTA had no appreciable affect on polyamine levels in either cell line. This was in contrast

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Table II. Effects of MTA and MFMTA on Growth and Polyamine Pools in L1210 and L5178Y Murine Leukemia Cells

cell line	treatment[a] (48 h)	concn, μM	growth, % of control	polyamine pools, nmol/10^6 cells		
				Put	Spd	Spm
L1210	none		100	0.62	3.22	0.73
	MTA (3)	600	53	3.64	1.59	0.20
	MFMTA (9)	200	67	0.62	2.93	0.60
L5178Y	none		100	0.32	1.94	1.30
	MTA (3)	60	57	1.24	3.03	0.47
	MFMTA (9)	200	45	0.41	2.47	1.32

[a] These data represent the average of at least three separate experiments. Because mean values for polyamine determinations varied between experiments, but not relative to control values within each experiment, standard deviations were not included.

to MTA which, at doses equitoxic in both cell lines, decreased Spm and, to a lesser degree, Spd pools at 48 h.

Conclusions. MFMTA and 5′-FMTA, two novel fluorinated analogues of MTA, have been synthesized. 5′-FMTA has been found to be rapidly degraded in aqueous medium, and as a consequence, its biological effects in cellular systems have been attributed to unidentified breakdown products. In contrast, MFMTA was found to be effective as a substrate and inhibitor of MTA phosphorylase. The initial characterization of MFMTA in L1210 and L5178Y cells has revealed it to be distinctly different from MTA in that its growth-inhibitory activity does not seem to be significantly affected by the presence or absence of MTA phosphorylase and it does not appear to be a product regulator of polyamine pathways. Since precise mechanisms by which MTA, itself, is growth inhibitory are uncertain, MFMTA, a minimally modified analogue of MTA, may serve as a useful probe in delineating sites of action separate from polyamine biosynthesis.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{19}F and ^1H NMR spectra were taken on a 60-MHz Varian EM-390 spectrometer by using tetramethylsilane as an internal standard. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. TLC was performed on EM aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm). 5′-Chloro-5′-deoxyadenosine (**2**),[11] 5′-deoxy-5′-(methylthio)adenosine (**3**),[11] and 5′-deoxy-5′-(methylthio)adenosine sulfoxide (**4**)[12] were obtained by literature procedures.

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-(methylthio)adenosine Sulfoxide (5). Acetic anhydride (2.2 mL, 23 mM) was added at room temperature to a suspension of **4** (3.0 g, 9.6 mM) and 4-(dimethylamino)pyridine (88 mg, 0.73 mM) in trimethylamine (3.5 mL, 25.3 mM) and acetonitrile (120 mL) according to the general procedure of Matsuda et al.[18] Initial clearing of the suspension was followed by formation of a new precipitate several minutes later. TLC (CH_2Cl_2/MeOH, 21:4) at 30 min indicated the absence of starting material. After 1 h the reaction mixture was evaporated to dryness in vacuo; to remove traces of acetic acid, toluene and methanol were added and the solution was evaporated. The residue was recrystallized from (methanol–$CHCl_2$)/(ether–petroleum ether) to give **5** (2.83 g, 74%) as white crystals: mp 226 °C dec; ^1H NMR ($CDCl_3$) δ 8.25 (2 s, 1 H, purine H), 7.86 and 7.80 (2 s, 1 H, purine H), 5.90 (m, 5 H, NH_2, H-1′, H-2′, H-3′), 4.73 (m, 1 H, H-4′), 3.32 (m, 2 H, $CH_2S{=}O$), 2.60 (2 s, 3 H, $CH_3S{=}O$), 2.18 (s, 3 H, $CH_3C{=}O$), 2.10 (s, 3 H, $CH_3C{=}O$). Anal. ($C_{15}H_{19}N_5O_6S$) C, H, N, S.

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-(methylthio)adenosine (7). O-Acetylation of MTA was carried out by using the same reaction conditions described for preparation of **5**. The product was recrystallized from CH_2Cl_2/ether/petroleum ether to give **7** (75%) as white crystals: mp 145 °C; ^1H NMR ($CDCl_3$) δ 8.30 (s, 1 H, purine), 7.96 (s, 1 H, purine H), 6.15 (s, 2 H, NH_2) 6.10 (d, 1 H, H-1′, J = 6 Hz) 5.92 (t, 1 H, H-2′, J = 6 Hz), 5.55 (t, 1 H, H-3′, J = 5.4 Hz), 4.35 (q, 1 H, H-4′, J = 5.4 Hz), 2.93 (d, 2 H, CH_2S, J = 5.4 Hz), 2.13 (s, 6 H, $CH_3C\Delta bdO$), 2.03 (s, 3 H, CH_3S). Anal. ($C_{15}H_{19}N_5O_5S$) C, H, N.

5′-Deoxy-5′-[(monofluoromethyl)thio]adenosine (9) and 5′-Deoxy-5′-(methylthio)adenosine (10). (A) McCarthy Transformation Using DAST. A dried, septum-fitted, one-necked flask containing **5** (2.0 g, 5.0 mM) in 11 mL of chloroform (freshly dried over alumina) under argon was injected with DAST (6.2 mL, 50 mM). The flask was then heated in an oil bath at 55 °C with stirring for 2.5–3 h, until TLC (CH_2Cl_2/MeOH, 23/2.5) indicated complete disappearance of starting material. The reaction mixture was carefully washed with 50% aqueous saturated sodium bicarbonate until the pH of the wash was basic. The separated organic layer was dried over $MgSO_4$, filtered, and evaporated to give a crude product containing 2′,3′-di-*O*-acetyl-5′-deoxy-5′-[(monofluoromethyl)thio]adenosine (**6**) and 2′,3′-di-*O*-acetyl-5′-deoxy-5′-fluoro-5′-(methylthio)adenosine (**8**). ^1H NMR ($CDCl_3$) of this product showed a doublet at δ 5.55 (J = 52.5 hZ), and ^{19}F NMR ($CFCl_3$) showed a triplet at δ (from $CFCl_3$) −182 (J = 54 Hz), both characteristic of **6**; ^{19}F NMR also showed a multiplet at δ (from $CFCl_3$) −163.5 (J = 2.5 Hz) characteristic of the diastereomeric **8**. The reaction residue containing **6** and **8** was dissolved in a minimum amount of methanol and added to a solution of methanol (220 mL) previously saturated with ammonia at −10 °C. The solution was allowed to stir at room temperature for 2.5 h at which time TLC (CH_2Cl_2/MeOH, 21.5:3.5) indicated disappearance of starting material. At this point the reaction flask could be stored overnight at dry ice temperature. Evaporation of the methanolic solution in vacuo gave a colored residue, which was chromatographed on Bio-Rad AG 1-X8 (OH^-) resin (200–400 mesh). The resin was prepared by conversion of Bio-Rad AG1-X8 (Cl^-) resin using 1 N aqueous NaOH, followed by a water wash and then a methanol flush. The residue was dissolved in methanol, applied to a short column (32 × 115 mm) of resin, and eluted exhaustively with methanol, a procedure that removed a substantial amount of dark colored impurities which were retained on the resin. The methanolic eluant was concentrated in vacuo and then applied to a second, long column (32 × 350 mm) of the resin. Elution with methanol resulted in separation of **9** and **10**.

In this manner, **9** [140 mg, 10.5% (yield based on **5**)] and **10** (416 mg, 31.3%) were obtained. Mixed fractions of **9** and **10** (67 mg, 5%) were pooled for subsequent refractionation. Compound **9**: ^1H NMR (Me_2SO-d_6) δ 8.30 (s, 1 H, purine H), 8.18 (s, 1 H, purine H), 7.20 (s, 2 H, NH_2), 5.85 (d, 2 H, H-1′, J = 6 Hz), 5.55 (d, 2 H, CH_2F, J = 52 Hz), 5.40 (m, 2 H, OH) 4.70 (t, 1 H, H-2′, J = 6 Hz), 4.10 (m, 2 H, H-3′, H-4′), 3.07 (m, 2 H, CH_2S); ^{19}F NMR (Me_2SO-d_6) δ (from $CFCl_3$) −183 (t, J = 51 Hz). Anal. ($C_{11}H_{14}N_5O_3SF$) C, H, N. Compound **10**: ^1H NMR (Me_2SO-d_6), δ 8.22 (s, 1 H, purine H), 8.12 (s, 1 H, purine H), 7.20 (s, 2 H, NH_2), 6.00 (d, $^1/_2$ H, CHF, J = 54 Hz), 5.96 (d, $^1/_2$ H, CHF, J = 54 Hz), 5.90, (2 d, 1 H, H-1′), 5.50 (m, 2 H, OH), 4.67 (m, 1 H, H-2′), 4.22 and 4.0 (2 m, 2 H, H-3′ and H-4′), 3.15 and 2.20 (d and 2 d, 3 H, CH_3S); ^{19}F NMR (Me_2SO-d_6) δ (from $CFCl_3$) −158.85 and −159 (2 d, J = 52.5 Hz), −162.75 and −163 (2 d, J = 52.5 Hz). (Note: The proton and fluorine NMR spectral patterns reflect the diastereomeric composition of **10**.) Anal. ($C_{11}H_{14}N_5O_3SF$) C, H, N, S.

(B) McCarthy Transformation Using meDAST. According to the procedure described in (A) but with meDAST replacing DAST, **5** (493 mg, 1.24 mM) was converted to **9** (54 mg, 14%), **10** (157 mg, 40%), and 5′-deoxy-5′-(methylthio)adenosine (**3**) (48

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mg, 13%). Compound **3** was identified by comparison of its ^1H NMR with that of authentic **3**. The ^1H NMR spectrum of an authentic sample of **7** was used to confirm its presence in the crude meDAST reaction mixture.

Biological Methods. [*methyl*-^3H]-*S*-Adenosylmethionine was purchased from Amersham Corp. (Arlington Heights, IL) and was converted to [*methyl*-^3H]MTA by the method of Schlenk et al.[19] Radiolabeled MTA was purified by TLC using acetone/ethyl acetate/H_2O (6:3:1) as solvent. 5′-Deoxy-5′-iodoadenosine (5′-IAdo) was purchased from Sigma Chemical Co. (St. Louis, MO).

MTA Phosphorylase. MTA phosphorylase was purified from mouse liver cytosol by acid precipitation to pH 5.0 with 1 M acetic acid. After removal of the precipitate by centrifugation, the supernatant was brought to pH 7.0 by titration with 0.5 M bicine, pH 9.2. The neutralized supernatant was heated to 65 °C and kept at this temperature for 2 min. After chilling, denatured protein was removed by centrifugation. The enzyme was then loaded on DEAE-Sephacel equilibrated with 10 mM HEPES, pH 7.4, and 1 mM dithiothreitol. The column was developed with a gradient of KCl in the above buffer and MTA phosphorylase eluted at ca 0.08 M KCl. Active fractions were pooled, concentrated with poly(ethylene glycol), and dialyzed against 10 mM HEPES, pH 7.4, and 1 mM dithiothreitol. This material, purified 43.5-fold with an 86% yield, was used as the source of enzyme.

Assay for MTA Phosphorylase Activity. Enzyme activity was determined by measuring the conversion of [*methyl*-^3H]MTA to [*methyl*-^3H]MTRP. The standard reaction mixture contained 4 μmol of potassium phosphate, pH 7.4, 0.1 μmol of dithiothreitol, 20 μg of bovine serum albumin, 50 nmol of MTA, and approximately 2×10^5 cpm [*methyl*-^3H]MTA in a final volume of 0.1 mL. After incubation at 37 °C for 10 min, the reaction was stopped by the addition of 0.1 mL of charcoal solution (1 g of norite, 0.5 g of dextran, 20 mL of 10% trichloroacetic acid). After 5 min on ice, the suspension was centrifuged and an aliquot of the clear supernatant was counted in a liquid scintillation spectrometer.

The ability of MFMTA and 5′-FMTA to act as substrates for MTA phosphorylase was tested by the spectrophotometric assay of Savarese et al.[20]

Cell Growth. Murine lymphocytic leukemia L1210 and L5178Y cell lines were grown in RPMI 1640 medium supplemented with 10% Nu Serum IV (Collaborative Research, Inc., Lexington, MA) and HEPES/MOPS buffer as previously described.[16] Cell cultures (0.3×10^5 cells/mL) were treated at 0 h with 5′-IAdo (Aldrich Chemical Co.), MFMTA, or MTA at concentrations up to 1000 μM and 5′-FMTA up to 100 μM. All compounds were dissolved in dimethyl sulfoxide and diluted in serum-free medium. After 48 h, cells were removed and cell number was determined by electronic particle counting (Cell-Dyne 300, Sequoia-Turner Corp., Mountain View, CA). The results were expressed as percent of cell growth in the absence of added inhibitors. The concentration that resulted in 50% growth inhibition (IC_{50} at 48 h) was determined.

Polyamine Pools. After cells were treated for 48 h with MFMTA or MTA at their approximate IC_{50} concentrations, a sample of cells was taken for polyamine determinations after extraction with 0.6 M perchloric acid (10^7 cells/0.5 mL of acid). The extract (50 μL) was analyzed by HPLC by using a system based on cation exchange and postcolumn derivatization with *o*-phthalaldehyde as described by Porter et al.[21]

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Registry No. **3**, 2457-80-9; **4**, 3387-65-3; **5**, 119771-16-3; **6**, 119771-18-5; **7**, 119771-17-4; (5*R*)-**8**, 119771-19-6; (5*S*)-**8**, 119771-20-9; **9**, 118560-47-7; (5*R*)-**10**, 119771-21-0; (5*S*)-**10**, 119771-22-1; MTA phosphorylase, 61970-06-7.

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18-Cycloalkyl Analogues of Enisoprost[1]

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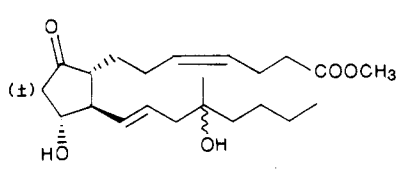
By use of standard cuprate methodology, a series of 18-cycloalkyl analogues of enisoprost was prepared in an effort to impede ω chain metabolism and prolong duration of gastric antisecretory activity. An initial product of ω chain oxidation, the C-20 hydroxy analogue, was also synthesized for pharmacological comparison. The cyclopropyl, cyclobutyl, and cyclopentyl analogues were approximately one-fourth as potent as enisoprost in inhibiting gastric acid secretion, while the cyclohexyl and cycloheptyl analogues showed very weak activity, and the 20-hydroxy compound was inactive at a dose 100 times the ED_{50} of enisoprost. The cyclobutyl compound had a longer duration of antisecretory action than enisoprost and the other cycloalkyl analogues. The cycloalkyl analogues unexpectedly possessed low diarrheogenic activity in rats.

Natural prostaglandins are susceptible to three major modes of metabolic inactivation: side chain hydroxyl oxidation, β-oxidation of the acid chain, and oxidation of the ω chain terminus. The oxidative events occur rapidly in animals and humans and have been a critical drawback for the potential therapeutic application of E-type prostaglandins to peptic ulcer disease.[2] Over the years the primary focus of our synthetic prostaglandin program has been the design of structural modifications that would prevent or slow these metabolic processes and thus provide analogues with an improved activity/duration profile compared to natural prostaglandins.

Enisoprost, **1**, is a 16-hydroxy prostaglandin analogue currently under clinical study for the treatment of peptic ulcer disease and related conditions.[3] In addition to a



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methyl group at C-16 to block the metabolic oxidation of the hydroxy moiety, enisoprost also contains a cis double bond at C-4,5 that reduces susceptibility to β-oxidation.[4]

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