Isozyme-Specific Enzyme Inhibitors. 13.[1] *S*-[5′(*R*)-[(*N*-Triphosphoamino)methyl]adenosyl]-L-homocysteine, a Potent Inhibitor of Rat Methionine Adenosyltransferases

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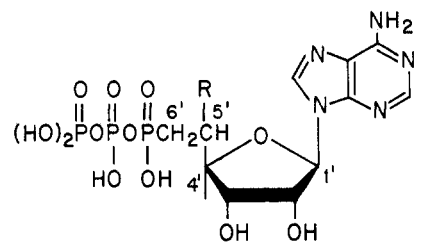
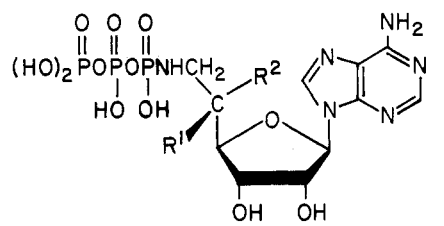
A synthesis is described of the title compound and its 5′*S* epimer, which are two-substrate adducts of adenosine 5′-triphosphate (ATP) and L-methionine (Met) in which the C(5′)H_2OP system in ATP is replaced by CH(R)CH_2NHP [R = L-S$(CH_2)_2$CH$(NH_2)CO_2$H]. The 5′*R* epimer was a potent nonselective competitive inhibitor [averaged K_i = 0.32 μM; K_M(ATP)/K_i = 440] vs. ATP of the rat M-2 (normal tissue) and M-T (Novikoff ascitic hepatoma) variants of methionine adenosyltransferase. It produced simple noncompetitive inhibition (averaged K_i = 2.7 μM) vs. Met with both variants. The 5′*S* epimer inhibited M-T competitively vs. ATP, but was 74-fold less effective than the 5′*R* epimer. Replacement of the homocysteine moiety in the 5′*R* epimer by hydrogen markedly reduced inhibitory potency, as indicated by K_i values of 14 μM for competitive inhibition vs. ATP and 580 μM for noncompetitive inhibition vs. Met with M-2. The data suggest that the 5′*R* epimer can interact simultaneously with two enzymic sites. Information on the kinetic mechanism of a human counterpart of M-2 and inhibitor properties of a previously studied Met-ATP adduct are consistent with the view that the two sites might resemble those that interact with the initial products of the reaction, *S*-adenosylmethionine and triphosphate.

Evidence outlined previously[2] indicates that isozyme-specific enzyme inhibitors could be useful in the derivation of new antineoplastic agents. Earlier studies in the present series examined several approaches to the design of such inhibitors. In part of that work, three enzyme-catalyzed reactions, each involving two substrates, were studied, and two-substrate adducts were synthesized that were linked covalently between the two atoms that become bonded in the respective reactions.[3-5] These adducts, either in unmodified form or after attachment of substrate substituents known to produce isozyme-selective inhibition, were found to inhibit two of the three enzyme-catalyzed reactions in a potent and isozyme-selective manner. Studies with this same class of two-substrate adduct were next extended to rat tissue variants of a fourth enzyme, ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) (MAT),[6] because a variant, M-T, predominant in rat ascitic hepatoma cells[7,8] is potentially useful as a target in attempted derivation of an isozyme-specific antineoplastic agent. MAT catalyzes attack of the sulfur of L-methionine on C5′ of adenosine 5′-triphosphate (ATP) to form *S*-adenosylmethionine. The two-substrate adducts initially synthesized were the two 5′ epimers of the 5′-*C*-L-homocystein-*S*-yl derivative of the phosphonate isostere of ATP (Scheme I, **15a,b**).[6] The more active epimer was a moderately effective inhibitor [K_M(ATP)/K_i = 4] of both the M-T form and a form (M-2) of MAT that predominates in most normal rat tissues.[9,10] Kinetic analysis of the inhibitions, together with other evidence, indicated that this epimer interacted simultaneously with the ATP and methionine binding sites of the two MAT forms.[6]

Scheme I



15a,b: R = $S(CH_2)_2CH(NH_2)CO_2H$



13a [5′(*R*)]: $R^1 = S(CH_2)_2CH(NH_2)CO_2H$, R^2 = H

13b [5′(*S*)]: R^1 = H, $R^2 = S(CH_2)_2CH(NH_2)CO_2H$

14: $R^1 = R^2$ = H

The above findings have encouraged us to study additional methionine–ATP adducts in a search for more potent inhibitors of the M-T form of MAT. Described here is the synthesis of the two 5′ epimers of a compound (**13a,b**) that differs from **15a,b** by the presence of an amino nitrogen between C6′ and P^α. The absolute C5′ configurations of **13a** and **13b** were determined by conversion of intermediates to known compounds. The ATP analogue **14**, in which the L-homocysteine residue of **13a,b** is replaced by hydrogen, was prepared also. All compounds were analyzed kinetically as potential dual substrate site inhibitors of both the M-T and the M-2 forms of MAT.

Chemical Syntheses. A 1:3 mixture of the 5′ epimers **2a** and **2b** of N^6-benzoyl-5′-*C*-(nitromethyl)-2′,3′-*O*-isopropylideneadenosine was obtained conveniently by a described base-catalyzed condensation of nitromethane with the corresponding 5′-aldehyde **1**.[11] Epimers **2a** and **2b** were separated by preparative HPLC on silica gel.

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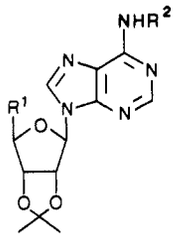
HPLC analysis showed that upon debenzoylation in methanol solution[12] **2a** gave the known 5′(*S*)-*C*-(nitromethyl)-2′,3′-*O*-isopropylideneadenosine[13] (**3a**) while **2b** gave the corresponding 5′*R* epimer **3b**. Catalytic reduction with platinum of the nitro groups of **2a,b**, like those of **3a,b**,[13] occurred readily in methanolic solution provided that relatively high amounts of catalyst were used. No reduction occurred when ethanol was the solvent. The resulting amines were isolated as their homogeneous *N*-Boc derivatives **4a,b** in 45% overall yield from **2a,b**. Treatment of **4a,b** in pyridine solution with *p*-toluenesulfonyl chloride then gave the respective *O*-tosyl derivatives **5a,b** in homogeneous form in 75–85% yield. In the present work the "a" series of compounds led to a stronger inhibitor (**13a**, see below) of MAT than the "b" series, but were stereochemically unfavored in the reaction of **1** with nitromethane. It was noted, however, that treatment of **5b** with KNO_2 in dimethyl sulfoxide[14] was effective in inverting the 5′ configuration and produced **4a** in 48% yield in a single trial.

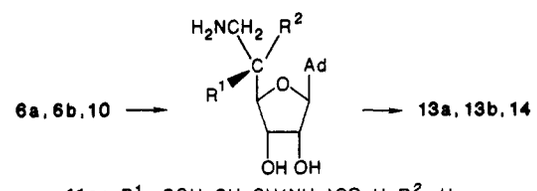


	R^1	R^2	C5′ configurations in the **a** and **b** series, respectively
1	CHO	Bz	
2a,b	$CH(OH)CH_2NO_2$	Bz	*S, R*
3a,b	$CH(OH)CH_2NO_2$	H	*S, R*
4a,b	$CH(OH)CH_2NH$-Boc	Bz	*S, R*
5a,b	$CH(OTs)CH_2NH$-Boc	Bz	*S, R*
6a,b	$CHCH_2NH$-Boc $SCH_2CH_2CH(NH_2)CO_2H$	H	*R, S*
7a,b	$CHNH$-Boc $CH_2SCH_2CH_2CH(NH_2)CO_2H$	H	*S, R*
8a,b	$CH(CH_2)$—N-Boc	Bz	*R, S*
9	$CH_2CH_2NH_2$	Bz	
10	$CH_2CH_2NH_2$	H	

Disodium L-homocysteinate did not react with **5b** in refluxing liquid ammonia under conditions in which it reacts with 2′,3′-*O*-isopropylidene-5′-*O*-tosyladenosine in a convenient synthesis of *S*-adenosyl-L-homocysteine.[15] Treatment of **5a** or **5b** in ethanol–toluene with excess of disodium L-homocysteinate and 18-crown-6, followed by removal under basic conditions of residual N^6-benzoyl groups from partially debenzoylated products, gave 10% yields overall of the desired 5′-(alkythio)-5′-deoxynucleosides **6a,b** together with 20% yields of the 5′-[(alkythio)methyl]-5′-deoxynucleosides **7a,b**. The low yields were associated with formation of several less polar UV-absorbing byproducts that were not identified. The structures of **6a,b** and **7a,b** were assigned from 300-MHz ^1H NMR spectra. The chemical shifts and multiplicity patterns of the 5′ and 6′ protons were in close agreement with those expected. Tosylates **5a,b** were concluded to produce **7a,b** via base-catalyzed formation of the *N*-Boc aziridines **8a,b**. These were too reactive to be detected among the products of the reaction, but could be isolated in 65–70% yield following treatment of **5a,b** with potassium *tert*-butoxide in *tert*-butyl alcohol. Under the conditions used to convert **5a,b** to a mixture of **6a,b** and **7a,b**, **8a,b** rapidly (in one-tenth the time) furnished a mixture of **7a** or **7b** and their N^6-benzoyl derivatives in high yield. Compounds **7a,b** could be prepared conveniently and more simply by reaction of disodium L-homocysteinate with **8a,b** in ethanol solution in the absence of 18-crown-6.

Since reaction of the aziridines **8a,b** with disodium L-homocysteinate furnished no **6a** or **6b** or their N^6-benzoyl derivatives, it appears that these 5′-thioethers arise exclusively from direct nucleophilic displacement of tosylate anion from the 5′-*O*-tosyl derivatives **5a** and **5b**, respectively. Conversion of **5a,b** to **6a,b** proceeded with no detectable racemization at C5′. Compounds **6a** and **6b** have been assigned C5′ configurations of *R* and *S*, respectively, on the assumption that transformation of **5a,b** to **6a,b** involves a single inversion at C5′. A double inversion could occur via the hypothetical intermediacy of an N3–C5′ cyclonucleoside formed from **5a,b**, but this appears unlikely because **5a,b** remained stable for more than 3 days under conditions that were the same as used for the 10-h conversion of **5a,b** to N^6-benzoylated **6a,b** except for replacement of the disodium L-homocysteinate by sufficient triethylamine to give the same apparent pH.

Treatment of **6a,b** with aqueous 90% trifluoroacetic acid removed the isopropylidene and butyloxycarbonyl groups to give the 5′-(alkylthio)-5′-(aminomethyl)-5′-deoxyadenosines **11a,b** in high yield. In minor modification of



11a: $R^1 = SCH_2CH_2CH(NH_2)CO_2H$, $R^2 = H$
11b: $R^1 = H$, $R^2 = SCH_2CH_2CH(NH_2)CO_2H$
12: $R^1 = R^2 = H$

conditions under which 5′-amino-5′-deoxyadenosine was converted into 5′-amino-5′-deoxyadenosine triphosphate (5′-amino-ATP),[16,17] **11a,b** were treated for 2 days at 22 °C with a saturated aqueous solution of trisodium trimetaphosphate maintained at pH 9.5–10 by additions of NaOH. The triethylammonium salts of the required *N*-triphosphoryl derivatives **13a,b** could be isolated quantitatively in homogeneous form in 60% yield by means of HPLC on C_{18} silica in MeOH–aqueous triethylammonium bicarbonate, which separated them from unchanged **11a,b**, sodium trimetaphosphate, and minor products that had the same retention time as adenosine 5′-diphosphate, and were concluded to be *N*-diphosphorylamino nucleosides homologous with **13a,b**. 5′-Amino-ADP likewise is a minor byproduct in the synthesis under similar conditions of 5′-amino-ATP and is slowly formed from 5′-amino-ATP at pH 10.[17] The adducts **13a,b**, isolated as their tetrasodium salts, were indistinguishable from each other and closely resembled adenosine 5′-triphosphate in their UV spectral, HPLC, paper chromatographic, paper electrophoretic, and ion exchange (PEI-cellulose) chromatographic properties. Like the previously described 5′-amino-ATP[16,17] and *N*-alkylphosphoramidates in general, **13a,b** were cleaved rapidly to the parent amines **11a,b**

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Table I. Physical Properties of Adenine Nucleoside and Nucleotide Derivatives

compd	R_f values from chromatography in solvent systems						electrophoresis, rel mobility		HPLC retention time,[a] min
	A	B	C	D	E	F	pH 4.5	pH 9.0	
2a			0.07	0.25		0.90			5.0[b]
2b			0.07	0.22		0.90			6.0[b]
3a									9.0[c]
3b									8.0[c]
4a				0.17	0.35				14.5[b]
4b				0.17	0.35				13.5[b]
5a				0.25	0.69				46.0[d,e]
5b			0.11	0.25	0.69				51.0[d,e]
6a	0.86	0.94		0	0	0.15			6.0[f]
6b	0.86	0.94		0	0	0.15			6.0[f]
7a	0.86	0.94		0	0	0.24			8.7[f]
7b	0.86	0.94		0	0	0.24			8.7[f]
8b			0.14[g]		0.53				10.5[h]
10						0.24	−1.7		
11a	0.24	0.50				0	−1.3		10.5,[i] 2.9,[j] 6.0[k]
11b	0.24	0.50				0	−1.3		10.5,[i] 2.9,[j] 6.0[k]
12	0.38	0.60				0.06	−1.7		10.0[l]
11a DP		0.29						0.87	26.0[k]
13a		0.29						0.87	16.7,[j] 32.0[k]
13b		0.29						0.87	16.7,[j] 32.0[k]
14	0.04	0.25						0.95	22.0[l]
AMP								1.67	6.2,[j] 14.5[k]
ADP		0.38						1.35	13.5,[j] 26.0[k]
ATP	0.09	0.38						1.0	17.6,[j] 33.0[k]

[a] All gradients were linear. Flow rates were 2 mL/min with a Waters C_{18} Nova-pak column unless otherwise stated. [b] 40–70% MeOH in H_2O (10 min). [c] 30–60% MeOH in H_2O (10 min). [d] MeOH–H_2O (6:4). [e] Waters μ-Bondapak C_{18} column. [f] MeOH–H_2O (11:13). [g] R_f of **8b** = 0.37 after three developments; R_f of **5b** = 0.28. [h] 40–100% MeOH in H_2O (10 min); t_R of **5b** = 10.8 min. [i] 0–50% MeOH in H_2O (10 min). [j] 0–30% MeOH in 0.1 M K_2HPO_4–0.025 M Bu_4NHSO_4 (pH 7.6) (30 min). [k] 0–2% MeOH in 0.5 M $Et_3NH{\cdot}HCO_3$ (pH 7.5) (30 min). [l] 2% MeOH–0.5 M $Et_3NH{\cdot}HCO_3$ (pH 7.5).

under mildly acidic aqueous conditions. HPLC analysis revealed that they were stable at room temperature for several hours at pH 8.2 under the conditions employed in the enzyme studies described below, which employed an assay period of 10 min at 37 °C.

In the synthesis of **14**, the known N^6-benzoyl-5′-deoxy-2′,3′-*O*-isopropylidene-5′-(nitromethyl)adenosine[11] was hydrogenated in the presence of platinum under the conditions used in the reduction of **2a,b**. The reduction proceeded at a rate similar to that with **2a,b** to furnish the amine **9** in admixture with minor amounts of the debenzoylated product **10**. Debenzoylation was completed by the action of NH_3–MeOH to give homogeneous **10** in 50% yield overall from the 5′-*C*-(nitromethyl) nucleoside derivative. Removal of the isopropylidene group of **10** to give **12** proceeded quantitatively under the action of aqueous 90% trifluoroacetic acid. Treatment of **12** with sodium trimetaphosphate for 4 h at pH 9 resulted in 77% conversion to the desired ATP analogue **14**; this was isolated as a sodium salt by the methods used in the preparations of **13a,b**. HPLC analysis showed that **14** was stable in the enzyme assay buffer at pH 8.2 for at least 1 h at 37 °C.

Enzyme Studies. Table III lists inhibition constants of **13–15** determined under conditions that were the same for M-2 and M-T and were used previously in studies with the adducts **15a,b**.[6] Adduct **13a** was a potent inhibitor of M-2 and M-T. As illustrated in Figure 1, it gave competitive kinetics vs. MgATP [K_M(ATP)/K_i = 390 and 520, respectively] and simple noncompetitive kinetics vs. L-methionine [K_M(Met)/K_i = 1.7 and 6.8, respectively]. The inhibition constants of **13a** were the same, within experimental error, for the two MAT forms. Adduct **13a** inhibited the two forms 115-fold more effectively than **15a** as judged by the inhibition constants obtained with variable ATP. MAT-catalyzed reactions have been shown to involve the intermediacy of enzyme-bound triphosphate which undergoes cleavage of what was originally the β,γ phosphoanhydride bond of ATP to give inorganic phosphate and pyrophosphate.[18] The sodium salt of the putative diphosphate analogue of **13a** did not inhibit M-T at a level of 20 μM, indicating that **13a** inhibits the two MAT forms directly and not after a hydrolysis to the corresponding diphosphate mediated by the tripolyphosphatase activities of these enzymes.

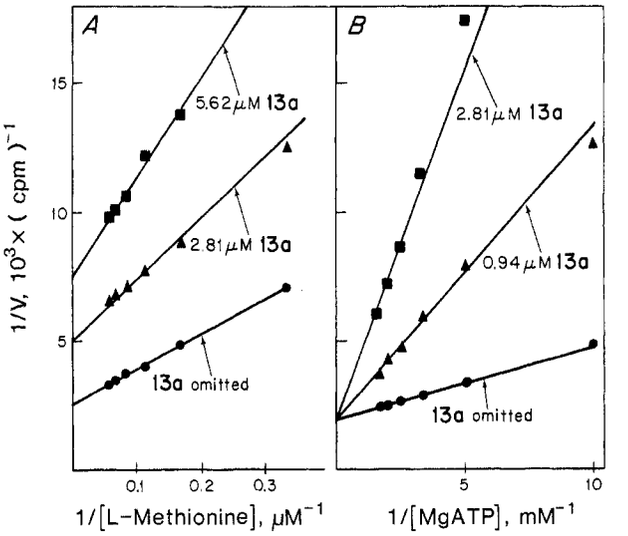

Figure 1. Inhibition of M-2 by **13a** with (A) variable L-methionine and 2 mM MgATP or (B) variable MgATP and 60 μM L-methionine. Other conditions are given in the Experimental Section.

The 5′ epimeric adduct **13b** also inhibited M-T competitively with respect to ATP but was 74-fold less effective than **13a** (Table III). HPLC analysis showed that during the studies of M-T inhibition **13b** underwent no detectable conversion to the corresponding diphosphate or to other

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Table II. ^{1}H NMR Data (300 MHz) for Adenine Nucleoside Derivatives[a]

	chemical shift, δ from $SiMe_4$ (multiplicity) [coupling constant, Hz]											
compd	H-2 or H-8	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	CMe_2	Boc C_4H_9	Boc N*H*	N*H* Bz	$NCOC_6H_5$	other protons
2a	8.01 (s), 8.75 (s)	5.95 (d) [2.0]	5.16 (m)	5.16 (m)	4.51 (br s)	4.59 (m)	4.49 (d) 6′A [3.2], 4.47 (d) 6′B [6.2]	1.63 (s), 1.37 (s)			*b*	8.01 (d) 7.55 (m)	
2b	8.11 (s), 8.75 (s)	5.96 (d) [4.2]	5.17 (dd) [5.9]	5.10 (dd) [1.8]	4.41 (t) [1.8]	4.75 (m)	4.56 (d) 6′A [5.8] 4.54 (d) 6′B [2.9]	1.63 (s), 1.37 (s)			*b*	8.01 (d) 7.55 (m)	
4a	8.01 (s), 8.72 (s)	5.94 (d) [4.7]	5.15 (t) [4.7]	5.04 (d) [4.7]	4.47 (br s)	3.88 (m)	3.33 (m) 6′A, 3.12 (m) 6′B	1.62 (s), 1.35 (s)	1.40 (s)	5.10	9.25 (s)	8.00 (d), 7.52 (m)	5.86 (d) [10.2], 5′-O*H*
4b	8.08 (s), 8.76 (s)	6.0 (d) [6.0]	5.18 (t) [6.0]	5.07 [1.3]	4.37 (br s)	4.03 (m)	3.45 (m) 6′A, 3.18 (m) 6′B	1.61 (s), 1.35 (s)	1.42 (s)	5.15	8.96 (s)	8.00 (d), 7.53 (m)	6.25 (br s), 5′-O*H*
5a	8.17 (s), 8.77 (s)	6.14 (d) [2.4]	5.17 (dd) [6.0]	5.07 (dd) [3.0]	4.39 (dd) [4.2]	5.0 (m)	3.49 (m) 6′A, 3.21 (m) 6′B	1.58 (s), 1.34 (s)	1.43 (s)	*b*	9.1 (s)	8.05 (d), 7.57 (m)	7.74 (d) [9.0], 7.24 (d) [9.0], $OSO_2C_6H_4$; 2.34 (s), OSO_2ArCH_3
5b	8.09 (s), 8.77 (s)	6.03 (d) [1.8]	5.29 (dd) [4.6]	5.08 (t) [4.6]	4.24 (t) [4.6]	5.0 (m)	3.34 (m) 6′A, 3.24 (m) 6′B	1.55 (s), 1.33 (s)	1.37 (s)	*b*	*b*	8.02 (d), 7.52 (m)	7.75 (d) [9.0], 7.25 (d) [9.0], $OSO_2C_6H_4$; 2.30 (s), OSO_2ArCH_3
6a	8.03 (s), 8.07 (s)	5.99 (d) [3.0]	5.38 (m)	5.18 (m)	3.96 (m)	2.80 (m)	3.10 (m)	1.40 (s), 1.20 (s)	1.01 (s)	*c*			3.60 (m), 2″; 2.55 (m), 4″; 1.80 (m), 3″;
6b	7.99 (s), 8.13 (s)	5.94 (d) [2.1]	5.23 (dd) [3.4]	5.00 (dd) [3.5]	4.25 (br s)	2.92 (m)	3.14 (d) [6.0]	1.45 (s), 1.16 (s)	1.24 (s)	*c*			3.30 (t) [6.0], 2″; 2.42 (t) [7.2], 4″; 1.71 (m), 3″;
7b	8.02 (s), 8.07 (s)	5.98 (d) [3.5]	5.28 (dd) [6.0]	4.87 (dd) [1.8]	4.32 (m)	3.80 (m)	2.5 (m)	1.46 (s), 1.23 (s)	1.20 (s)	*c*			3.54 (t) [6.2], 2″; 2.5 (m), 4″; 1.9 (m), 3″
8b	8.79 (s), 9.05 (s)	6.30 (d) [3.0]	5.22 (dd) [6.0]	5.13 (dd) [3.0]	4.37 (t) [3.0]	2.7 (m)	2.31 (d) 6′A [6.0] 2.16 (d) 6′B [3.0]	1.64 (s), 1.39 (s)	1.40 (s)		8.42 (s)	8.00 (d) 7.50 (m)	
11a	8.24 (s), 8.30 (s)	5.93 (d) [5.3]	4.72 (t) [5.3]	4.49 (t) [5.3]	4.05 (dd) [4.7]	3.01 (dt) [10.4]	3.2 (m)						3.67 (t) [7.3], 2″; 1.92 (q) [7.3], 3″; 2.54 (m), 4″;
11b	8.27 (s), 8.37 (s)	5.96 (d) [3.5]	4.61 (dd) [5.8]	4.49 (t) [5.8]	4.2 (dd) [3.0]	3.11 (m)	3.26 (m)						3.27 (m), 2″; 2.64 (m), 3″; 2.03 (m), 4″

[a] Solvents are given in the Experimental Section. [b] Not assignable. [c] Spectra obtained from solutions in D_2O.

Table III. Inhibition Constants of Adenine Nucleotide Derivatives with Kidney (M-2) and Novikoff Ascitic Hepatoma (M-T) Forms of Rat Methionine Adenosyltransferase[a]

	M-2: K_i,[b] μM (type of inhibn)		M-T: K_i, μM (type of inhibn)	
compd	ATP varied	Met varied	ATP varied	Met varied
13a[c]	0.36 (C)	3.0 (NC)	0.27 (C)	2.3 (NC)
13b[c]			20 (C)	
14	14 (C)	580 (NC)		
15a[c]	39 (C)[d]	62 (C)[d]	32 (C)[d]	
15b[c]	180 (C)[d]	200 (C)[d]	148 (C)[d]	

[a] When methionine (Met) was the variable substrate, [MgATP] was 2 mM with both MAT forms; with variable MgATP, [Met] was 60 μM with MAT-2 and 120 μM with MAT-T. For other conditions, see the Experimental Section. [b] C = competitive; NC = simple noncompetitive (the inhibitor reduces V_{max} and does not change K_M). [c] The absolute C5′ configurations are known for **13a,b** (see text) but not for **15a,b**. [d] Data from ref 6.

compounds. The stereospecificity of inhibition by **13a** and **13b** is seen from Table III to be higher than that of the previously studied adducts **15a** and **15b**.

Removal of the homocysteine residue of **13a**, giving **14**, increased the inhibition constant with M-2 by a factor of 40 with variable ATP and a factor of 190 with variable methionine (Table III), indicating a substantial contribution of the homocysteine residue to the affinity of **13a** for M-2. The magnitude of this contribution, together with the potent competitive inhibition exhibited by **13a** with respect to ATP, is consistent with a dual-site type of interaction with the enzymes. Among sites possibly involved are those for ATP, methionine, *S*-adenosylmethionine, or triphosphate. Of these, binding to the methionine site may not make a major contribution because of the noncompetitive character of the inhibition by **13a** with respect to methionine and because evidence suggests that M-2 and M-T may have little preference in the order in which they bind ATP and methionine.[6] It can be speculated that **13a** might interact simultaneously at sites that are similar or identical with those that bind tripolyphosphate and *S*-adenosylmethionine. Consistent with this is the noncompetitive inhibition with respect to methionine shown by either tripolyphosphate or *S*-adenosylmethionine with a human lymphocyte MAT that was concluded to be a counterpart of the M-2 variant of rat MAT.[19] The properties of **15a** have been taken to indicate that it could interact simultaneously with the ATP and methionine sites of M-2 in producing its inhibitory effects.[6] Possibly, therefore, introduction of an extra atom between P^{α} and C6′ of **15a** to give **13a** has simulated the C5′–O5′ cleavage that occurs in MAT-catalyzed reactions and has established between the tripolyphosphoryl and adenosylmethionyl moieties of **13a** a spatial relationship similar to that between enzyme-bound tripolyphosphate and adenosylmethionine in a ternary complex that is thought to form during the catalytic cycle.[18]

In summary, the P^{α}–ribose bridge segment of a previously described L-homocysteine–ATP S–C5′ adduct[6] has been lengthened by introduction of a single amine nitrogen. This caused the inhibitory potency $[K_M(ATP)/K_i]$ toward two forms of rat methionine adenosyltransferase to increase 115-fold and produced a potent inhibitor. One enzyme form tested, M-T, is of interest as a model mammalian target in cancer chemotherapy. The present inhibitor showed no significant selectivity for M-T with respect to M-2, the form predominant in most normal rat tissues. By structural modification, however, it may be possible to impart this selectivity while retaining inhibitory effectiveness. Studies of potential M-T inhibitors are continuing.

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Experimental Section

Chemical Synthesis. General Procedures. CH_3NO_2 and PtO_2 ("amorphous") were purchased from Aldrich Chemical Co. Di-*tert*-butyl pyrocarbonate and CF_3CO_2H were purchased from Sigma. *p*-Toluenesulfonyl chloride was recrystallized from hexane. Trisodium trimetaphosphate hexahydrate was recrystallized to homogeneity (TLC on PEI cellulose (Merck) in 1.2 M LiCl, R_f 0.6) from EtOH-H_2O.[20] *S*-Benzyl-L-homocysteine was prepared and purified as described.[21] Dimethyl sulfoxide (Me_2SO) and pyridine were distilled over CaH_2 and stored over molecular sieves. Paper chromatography was carried out by the descending technique on Whatman No. 1 paper and on Eastman cellulose TLC sheets in (A) 2-propanol–NH_4OH–water (6:3:1) and (B) 2-propanol–NH_4OH–water (6:1:3). Thin-layer chromatography (TLC) was run on Merck silica gel 60F-254 plates in (C) $CHCl_3$–MeOH (99:1), (D) $CHCl_3$–MeOH (97:3), (E) CH_3COOEt, and (F) $CHCl_3$–MeOH–4% CH_3COOH (lower layer) (3:2:1). Flash chromatography[22] was carried out with Merck silica gel (230–400 mesh) and with Baker bonded phase octadecyl silica. HPLC was performed on a Waters Model 204 chromatograph equipped with a dual solvent-delivery system and a Waters RCM-100 radial compression unit containing a 4 μm (Nova-Pak) or 10 μm particle size C_{18} cartridge, 8 mm × 10 cm. Semipreparative HPLC employed a C_{18} Chrompack column (250 × 12 mm) of Lichrosorb 10RP18 and a HPLC Technology Ltd. 5-μm Techsphere silica column (25 cm × 22.5 mm). Preparative chromatography employed a Waters Prep LC/System 500A and two silica cartridges. Electrophoresis was carried out on Whatman No. 1 paper at pH 4.5 (0.05 M NaOAc) or at pH 9.0 [0.05 M $Et_3NH{\cdot}HCO_3$–$(Et_3N)_2CO_3$]. UV spectra were obtained with a Cary Model 15 spectrophotometer and ^1H NMR spectra with a Nicolet NT-300 spectrometer. Chemical shifts are given as ppm (δ) downfield from $SiMe_4$; in D_2O solutions they are downfield from $Me_2SiCH_2CH_2SO_3Na$. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN, and were within ±0.4% of the theoretical values. Compounds for analysis were dried at 25 °C.

***N*⁶-Benzoyl-5′(*S*- and -*R*)-*C*-(nitromethyl)-2′,3′-*O*-isopropylideneadenosines (2a and 2b, Respectively).** *N*⁶-Benzoyl-2′,3′-*O*-isopropylideneadenosine[23] (9.8 g, 24 mmol) was converted to **1** as described.[24] After the reaction was complete, dicyclohexylurea was filtered off, and the filtrate was extracted with cyclohexane (4 × 100 mL) to remove excess of *N*,*N*-dicyclohexylcarbodiimide. The Me_2SO solution was diluted with chloroform (400 mL) and extracted with water (2 × 200 mL). The dried (Na_2SO_4) chloroform solution was evaporated in vacuo and benzene (100 mL) was added and evaporated to dryness in vacuo. The residual crude **1** was converted to **2a** and **2b** by a described procedure.[11] The crude product was subjected to flash chromatography[22] on silica gel with acetone–CH_2Cl_2 (1:9), giving a pale yellow gum (4.5 g, 40% yield) containing **2a,b** in 1:3 ratio as indicated by TLC and HPLC. A portion (2 g) of this was subjected to preparative HPLC using $CHCl_3$–MeOH (99:1) and three recycles to give homogeneous **2a** (0.5 g) and **2b** (1.08 g) as white foams: ^1H NMR ($CDCl_3$), see Table II.

Solutions of **2a** or **2b** (2 mg) in MeOH (200 μL) were stored at 20 °C for 72 h. HPLC (Table I) of the solutions either alone or coinjected with authentic **3a** or **3b**[13] showed that **2a** had been converted to **3a** and **2b** converted to **3b**.

***N*⁶-Benzoyl-5′(*S*- and -*R*)-*C*-[[*N*-(*tert*-butyloxycarbonyl)amino]methyl]-2′,3′-*O*-isopropylideneadenosines (4a and 4b, Respectively).** Compound **2a** (1.85 g, 3.94 mmol)

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was added to a suspension of prereduced PtO_2 (925 mg) in MeOH (45 mL). This was shaken at 20 °C and 50 psi of H_2 for 16 h, when TLC (solvent F) showed no starting material and two ninhydrin-positive spots of R_f 0.35 [UV_{max} (EtOH) 280 nm] and R_f 0.15 [UV_{max} (EtOH) 260 nm]. Electrophoresis at pH 4.5 indicated each compound was monocationic. The catalyst was filtered off and the filtrate was evaporated in vacuo. To a solution of the residue in CH_2Cl_2 (45 mL) was added di-*tert*-butyl pyrocarbonate (2.91 g, 13 mmol). TLC (solvents D, E, and F; Table I) showed the reaction was complete after 1 h, 20 °C. Solvent was evaporated in vacuo and the residue was partitioned between EtOAc (50 mL) and H_2O (30 mL). The EtOAc was dried (Na_2SO_4) and then evaporated in vacuo. The residue was purified by silica gel flash chromatography with EtOAc as eluant. Fractions containing **4a** were combined and evaporated in vacuo. After drying at 60 °C (0.5 mmHg) for 1 h, **4a** (1.04 g, 49%) was obtained as a pale yellow foam which was homogeneous by TLC and HPLC (Table I): UV_{max} (EtOH) 280 nm (ϵ 18 400); ^1H NMR ($CDCl_3$), see Table II.

Compound **2b** (2.5 g, 5.32 mmol) gave homogeneous **4b** (1.22 g, 43%) as a white foam under the above conditions: UV_{max} (EtOH) 280 nm (ϵ 18 700); ^1H NMR ($CDCl_3$), see Table II. Anal. ($C_{26}H_{32}N_6O_7 \cdot 1.0H_2O$) C, H, N.

N^6-Benzoyl-5′(*S*- and -*R*)-*C*-[[*N*-(*tert*-butyloxycarbonyl)amino]methyl]-2′,3′-*O*-isopropylidene-5′-*O*-(*p*-tolylsulfonyl)adenosines (5a and 5b, Respectively). A solution of **4a** (782 mg, 1.45 mmol) and *p*-toluenesulfonyl chloride (829 mg, 4.35 mmol) in pyridine (5 mL) was stored under argon at 20 °C for 28 h. Pyridine was removed by evaporation in vacuo. A solution of the dark residue in EtOAc (50 mL) was washed with saturated $NaHCO_3$ (3 × 20 mL) and then water (2 × 20 mL). The dried (Na_2SO_4) solvent was evaporated in vacuo, and the residue was purified by flash chromatography[22] on silica gel with EtOAc–hexane (3:1) to give **5a** (756 mg, 75%) as a pale yellow foam, homogeneous by TLC and HPLC (Table I): UV_{max} (EtOH) 280 nm (ϵ 19 104); ^1H NMR ($CDCl_3$), see Table II.

Compound **4b** (942 mg, 1.74 mmol) under the above conditions gave homogeneous **5b** (1.03 g, 85%) as a pale yellow foam: UV_{max} (EtOH) 280 nm (ϵ 18 772); ^1H NMR ($CDCl_3$), see Table II. Anal. ($C_{33}H_{38}N_6O_9S \cdot 1.5H_2O$) C, H, N, S.

Preparation of 4a from 5b. A suspension of KNO_2 (750 mg, 8.8 mmol) in benzene (60 mL) was evaporated to dryness in vacuo. To this KNO_2 was added **5b** (215 mg, 0.31 mmol) and Me_2SO (8 mL). The mixture was stirred at 85–90 °C for 12 h. Water (200 mL) was added to the orange solution and the mixture was extracted with EtOAc (3 × 40 mL). The EtOAc solution was washed with brine (50 mL), then dried (Na_2SO_4), and evaporated in vacuo. Purification by silica gel flash chromatography using EtOAc–hexane (4:1) as eluent gave a white gum (81 mg, 48%) identical with **4a** prepared from **2a** by TLC and HPLC (Table I).

5′(*S*)-[[*N*-(*tert*-Butyloxycarbonyl)amino]methyl]-5′-deoxy-5′-L-homocystein-*S*-yl-2′,3′-*O*-isopropylideneadenosine (6b) and 5′(*R*)-[*N*-(*tert*-Butyloxycarbonyl)amino]-5′-deoxy-5′-(L-homocystein-*S*-ylmethyl)-2′,3′-*O*-isopropylideneadenosine (7b) and Their Epimers 6a and 7a. Sodium (497 mg, 21.6 mmol) was added in portions to a refluxing solution of *S*-benzyl-L-homocysteine (2.43 g, 10.8 mmol) in liquid NH_3 (120 mL) until the blue color persisted for 1–2 min. The color was discharged by addition of *S*-benzyl-L-homocysteine. NH_3 was evaporated off and the residue was dried at aspirator vacuum for 10 min. Argon was admitted. To the white powder were added degassed EtOH (21 mL), 18-crown-6 (471 mg, 2.16 mmol), and a solution of **5b** (750 mg, 1.08 mmol) in toluene (21 mL). The pale yellow solution was stored at 20 °C for 10 h. Solvents were removed in vacuo, and after trituration with hexane (3 × 20 mL) the residue was dissolved in MeOH–H_2O (1:1). The pH was adjusted to 9 with solid CO_2 and finally to 7 with 1 N HCl (3 mL) with stirring at 0 °C. The white suspension was filtered and the filtrate was saturated with NH_3 at 0 °C and stored at 4 °C for 1 h. Solvents were removed in vacuo, and the residual gum was suspended in MeOH–H_2O (1:1, 10 mL) and the pH was adjusted to 7 with 1 N HCl. The suspension was filtered and the filtrate was concentrated to 5 mL and applied to C_{18} silica gel (60 g) in a sintered funnel. Elution was carried out at reduced pressure with MeOH–H_2O (1:1, 8 × 70 mL). Fractions 2 and 3 containing **6b** and **7b** (5500 OD_{260} units, 34%) (OD_{260} units = volume in milliliters × optical density at 260 nm) were combined, concentrated to a small volume, and chromatographed on a C_{18} silica gel column (1.9 × 15 cm) applying gradients of 40%, 50%, and 60% MeOH in water (70 mL each) and a positive pressure of 7 psi of N_2. Compounds **6b** (1995 OD_{260} units, 12%) and **7b** (3800 OD_{260} units, 23%) were obtained as colorless gums homogeneous by TLC and HPLC (Table I): ^1H NMR (D_2O), see Table II.

Compound **5a** (125 mg, 0.18 mmol) gave **6a** (7% yield) and **7a** (12% yield) as colorless gums by the conditions described above. They were homogeneous by TLC and HPLC (Table I). Resonance assignments of **6a,b** and **7b** (Table II) were confirmed by systematic decoupling of all sugar protons. Anal. (**6b**) ($C_{23}H_{35}N_7O_7S \cdot 4H_2O$) C, N; H: calcd, 6.88; found, 6.46. Anal. (**7b**) ($C_{23}H_{35}N_7O_7S \cdot 2H_2O$) H, N, S; C: calcd, 46.85; found, 47.29.

N^6-Benzoyl-5′(*S*)-5′-[*N*-(*tert*-butyloxycarbonyl)amino]-2′,3′-*O*-isopropylidene-5′,5′-*C*,*N*-methyleneadenosine (8b). A solution of 0.1 M *t*-BuOK (2.9 mL) prepared from K and *t*-BuOH was added dropwise to a stirred solution of **5b** (101.5 mg, 0.146 mmol) in *t*-BuOH (0.5 mL) at 20 °C. After 1 h, the yellow suspension was saturated with CO_2, diluted with dichloromethane (2 mL), and centrifuged. The centrifugate was evaporated in vacuo. The residue was suspended in EtOAc (2 mL) and centrifuged. The pale yellow centrifugate was evaporated in vacuo. A solution of the residue in MeOH–$CHCl_3$ (1:99) (0.3 mL) was injected in three portions onto a semipreparative silica gel HPLC column which was eluted at 9 mL/min with the same solvent to give **8b** (40 mg, 67%) and **5b** (24 mg) as pale yellow foams homogeneous by TLC and HPLC (Table I): UV_{max} (EtOH) 280 nm; ^1H NMR ($CDCl_3$), see Table II. The resonances of H5′ and H6′ are in agreement with those of related aziridines.[25,26] Resonance assignments were confirmed by decoupling of sugar protons. Anal. ($C_{26}H_{30}N_6O_6 \cdot 1.05CHCl_3$) C, H, N, Cl.

Preparation of 7b from 8b. To **8b** (46 mg, 85.6 μmol) was added a 0.075 M solution of disodium L-homocysteinate in EtOH (133 μL, 1.2 equiv), and the pale yellow solution was stored at 20 °C for 6 h. Solvent was evaporated in vacuo and the residue was dissolved in methanolic NH_3 and stored at 4 °C for 1 h. Solvents were removed in vacuo and the residue was dissolved in MeOH–H_2O (1:1, 8 mL). The pH was adjusted to 7 with 1 N HCl and the resulting suspension was centrifuged. The centrifugate was purified by chromatography on a C_{18} silica gel column as described for the purification of **6b** and **7b**. Fractions containing **7b** were combined and evaporated in vacuo to give **7b** as a colorless gum (1000 OD_{260} units, 46% yield from **5b**) which was homogeneous by TLC and HPLC (Table I). The ^1H NMR (D_2O) spectrum was identical with that of **7b** prepared from **5b**.

5′-(Aminomethyl)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (10). N^6-Benzoyl-5′-deoxy-5′-(nitromethyl)-2′,3′-*O*-isopropylideneadenosine (230 mg, 0.5 mmol), obtained from **2a,b** as described,[11] was added to a suspension of prereduced PtO_2 (115 mg) in MeOH (5 mL) and hydrogenation was carried out as described above. TLC (solvent F) showed no starting material and two ninhydrin-positive spots of R_f 0.42 (compound **9**) and 0.24 (**10**) in about 7:3 ratio, respectively, together with several other products that were ninhydrin-negative. The suspension was filtered through Celite and MeOH was removed in vacuo. The residue was dissolved in AcOH–H_2O (1:4, 25 mL) and extracted with ether (2 × 25 mL). The aqueous phase was evaporated in vacuo and the residue was dried by evaporation with toluene (3 × 20 mL). A solution of the resulting gum in MeOH (5 mL) was cooled in ice and saturated with NH_3 gas. The mixture was stored at 4 °C for 18 h. Evaporation of solvents in vacuo, followed by evaporation with EtOH (2 × 10 mL), gave **10** (50% yield by spectrophotometry) as a gum which was homogeneous by TLC and electrophoresis (Table I).

5′-(Aminomethyl)-5′-deoxyadenosine (12). A solution of **10** (2560 OD_{260} units, 0.17 mmol) in aqueous 90% CF_3CO_2H (10 mL) was stored at 20 °C for 10 min. The CF_3CO_2H was immediately removed in vacuo. Toluene (2 × 20 mL) and then EtOH (20 mL)

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were successively added and evaporated from the residue to give the trifluoroacetate salt of **12** (100% yield spectrophotometrically) as a colorless gum which was homogeneous by cellulose TLC in *n*-BuOH–AcOH–H_2O (4:1:1), R_f 0.51 (R_f 0.74 for **10**), and 2-propanol–15% NH_4OH (7:3), R_f 0.50 (R_f 0.89 for **10**), and on paper electrophoresis (Table I). Anal. ($C_{13}H_{17}F_3N_6O_5 \cdot 0.5C_2H_5OH \cdot 1.5H_2O$) C, H, N.

5′(*S*)-(Aminomethyl)-5′-deoxy-5′-L-homocystein-*S*-yladenosine (11b). Compound **6b** was converted to **11b** as described for the synthesis of **12**. The product was chromatographed on a C_{18} silica gel column (1.9 × 15 cm) with MeOH–H_2O (2:3) as eluent to give **11b** as a colorless gum (40 μmol) which was homogeneous by TLC and HPLC analysis (Table I): ^1H NMR (D_2O), see Table II.

Compound **6a** (117 μmol) gave **11a** (100 μmol) as a colorless gum under the above conditions; **11a** was homogeneous by TLC and HPLC analysis (Table I); ^1H NMR (D_2O), see Table II.

5′(*S*)-[(*N*-triphosphoamino)methyl]-5′-deoxy-5′-L-homocystein-*S*-yladenosine (13b). Aqueous $Na_3P_3O_9 \cdot 6H_2O$ (120 μL of 0.6 M) was added to **11b** (39 μmol) and the solution was adjusted to pH 9.5–10 with 1 M NaOH (5 μL). Aliquots (7 × 5 μL) of 1 M NaOH were added periodically over 120 h to maintain the pH at 9.5–10. Aqueous $Et_3NH \cdot HCO_3$ (1 mL of 0.5 M) was added, and the solution was applied to a C_{18} silica gel column (0.8 × 15 cm) preequilibrated with 0.5 M $Et_3NH \cdot HCO_3$ (45 mL) at 4 °C. The column was eluted at 4 °C with 0.5 M $Et_3NH \cdot HCO_3$ and then with 2%, 5%, and 10% MeOH, respectively, in 0.5 M $Et_3NH \cdot HCO_3$ (30 mL each) by applying a positive pressure of N_2 and collecting 2–3-mL fractions. Fractions were analyzed by HPLC in 0.1 M K_2HPO_4–0.025 M Bu_4NHSO_4 (pH 7.5) with a linear gradient of 0–30% MeOH over 30 min. $Na_3P_3O_9$ eluted in the first fractions. Fractions containing **13b** (10 μmol) were evaporated in vacuo, and to the residue were successively added EtOH (2 × 5 mL) and MeOH (5 mL) and evaporated. The residue was dissolved in MeOH (1 mL), and 1 M NaI in MeOH (130 μL) was added and the tetrasodium salt was isolated as previously described[5] to give **13b** (4 mg, 4.8 μmol) as a white powder, UV_{max} (pH 8.5) 260 nm, homogeneous by TLC, HPLC (Table I), PEI-cellulose TLC in 1.2 M LiCl containing 0.05 M $NaHCO_3$ (R_f 0.60; ATP, 0.60), and paper chromatography in *n*-PrOH–NH_4OH–H_2O (55:10:35) (R_f 0.30; ATP, 0.40). Anal. ($C_{15}H_{22}N_7SP_3O_{14}Na_4 \cdot 4H_2O \cdot 0.5CH_3OH$) C, H, N.

Compound 13a, prepared from **11a** in 32% yield by the above method, was indistinguishable from **13b** in the systems of Table I and in the PEI-cellulose and the *n*-PrOH–NH_4OH systems above.

5′-[(*N*-Triphosphoamino)methyl]-5′-deoxyadenosine (14). To the trifluoroacetate salt of **12** (0.17 mmol) was added 0.6 M $Na_3P_3O_9 \cdot 6H_2O$ (670 μL). The solution was adjusted to pH 9.0 (with 0.1 M NaOH) and was maintained at pH 9.0 by additions of 0.1 M NaOH. After 4 h HPLC (2% MeOH in 0.5 M $Et_3NH \cdot HCO_3$) showed 77% conversion of **12** to **14** and minor amounts of several other UV-absorbing products. Compound **14** was obtained as its triethylammonium salt by column chromatography over C_{18} silica gel and converted to its sodium salt (0.06 mmol) by the methods used with **13b**: UV_{max} (pH 8.5) 260 nm. Other physical properties are given in Table I.

Enzyme Studies. M-2 and M-T preparations were obtained as described previously[27] except that the isolation and concentration of M-2 (final volume 0.14 mL/g of kidney extracted) and M-T (final volume 0.27 mL/g of Novikoff ascitic hepatoma cells extracted) were completed within 1 day.

Enzyme assays were conducted for 10 min at 37 °C in a final volume of 0.1 mL containing 150 mM KCl–15 mM $MgCl_2$–5 mM dithiothreitol–50 mM Tris·HCl, pH 8.2.[7] Each mixture was made up in duplicate. L-[*methyl*-^{14}C]Methionine (New England Nuclear Co., 54 Ci/mol) and MgATP were included at the levels specified below and in Table III, footnote *a*. A working enzyme solution was prepared freshly each day by 10-fold dilution of a stock solution.[27] Reactions were started by addition of 10 μL of working enzyme solution [(9.5–10.5) × 10^{-6} units of activity; 1 unit gives a V_{max} with 2 mM ATP of 1 μmol of product per min] and terminated by addition of 10 μL of 4 N $HClO_4$–10 mM L-methionine after immersing the solution in an ice bath. Each suspension was centrifuged and 50 μL of supernatant was applied to a 2.3-cm disk of phosphocellulose paper. Disks were washed as described,[28] then immersed in a toluene solution of phosphors, and counted in a Packard liquid scintillation spectrometer (Model 2425). Controls were provided by incubations carried out in the absence of ATP. Reaction velocities were linear for at least 30 min and were proportional to the amount of enzyme added at the levels of enzyme activity employed.

Inhibition studies were made with six to eight levels of MgATP or L-methionine in the range (0.5–4.0) × K_M for each of two inhibitor levels that were in the range (1–10) × K_i and for control mixtures lacking inhibitor. Inhibitors were dissolved in the above pH 8.2 buffer solution prior to testing. Inhibition constants (K_i values) were obtained to within ±15% from replots of inhibitor concentrations vs. slopes or intercepts on the vertical axis of double-reciprocal plots of velocity vs. substrate level. All of the latter plots were linear, as were the replots.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute and by grants to the Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania). We thank Dr. Sidney Weinhouse, Temple University, for kindly providing the rat Novikoff ascitic hepatoma cells from which the M-T preparations were obtained.

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Antisecretory Activity of Human, Dog, and Rat Metabolites of Fenoctimine

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Fenoctimine (**1a**), a nonanticholinergic inhibitor of gastric acid secretion in dogs and rats, was evaluated as a gastric antisecretory agent in humans. In humans it exhibited weak antisecretory activity and caused anticholinergic-like side effects such as dry mouth and nasal passages. Studies of the metabolic fate of fenoctimine in humans, dogs, and rats provided structures of the resultant metabolites. These were synthesized and tested for antisecretory and anticholinergic activity. The human metabolites were all less active than fenoctimine as antisecretory agents, and some displayed significant anticholinergic activity. These results suggest that the unexpectedly weak effect of fenoctimine as a gastric antisecretory agent in humans, as well as anticholinergic effects, may be due to its extensive metabolism, which is different from that seen in dog and rat.

Fenoctimine (**1a**) is active as a gastric antisecretory agent in animals[1-3] by an undetermined mechanism of action. In vitro studies on guinea pig ileum and atria[1] indicated that it was not a histamine H_2 antagonist, while its failure

0022-2623/87/1830-0894$01.50/0