

uct precipitated with ether. The hygroscopic yellow hydrochloride salt was suspended in benzene and the water was removed by azeotropic distillation for 2 hr with a trap. The material was no longer hygroscopic. Recrystallization from methanol-ether gave a white hydrated salt, mp 230–232°. *Anal.* (C₂₅H₂₁ClN₄·3HCl·0.5H₂O) C, H, N.

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Potential Inhibitors of S-Adenosylmethionine-Dependent Methyltransferases. 1. Modification of the Amino Acid Portion of S-Adenosylhomocysteine

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Structural analogs of S-adenosyl-L-homocysteine (L-SAH), with modifications of the amino acid portion of the molecule, have been synthesized and their abilities to inhibit catechol O-methyltransferase, phenylethanolamine N-methyltransferase, histamine N-methyltransferase, and hydroxyindole O-methyltransferase have been investigated. The data from these inhibition studies have resulted in a delineation of the structural features of SAH which are required for enzymatic binding of this ligand. In general, it was concluded that the terminal amino group, the terminal carboxyl group, and the sulfur atom of the homocysteine portion of SAH are required for maximum binding of SAH by these enzymes. The L configuration of the asymmetric amino acid carbon of SAH is generally required to produce maximum inhibition. The exception appears to be the potent inhibition of histamine N-methyltransferase by D-SAH. D-SAH was substantially less effective as an inhibitor of the other enzymes tested. The significance of these data relative to the nature of the SAH binding sites is discussed.

Since the discovery of S-adenosyl-L-methionine¹ (SAM),† a great variety of SAM-dependent biological transmethylation reactions have been demonstrated.² A general feature of many SAM-dependent methyltransferases is the inhibition produced by the demethylated product, S-adenosyl-L-homocysteine (L-SAH). This product inhibition by L-SAH suggests not only a new class of potential inhibitors for methyltransferases but also suggests a possible biological regulatory mechanism. Evidence to support a L-SAH mediated regulatory mechanism was re-

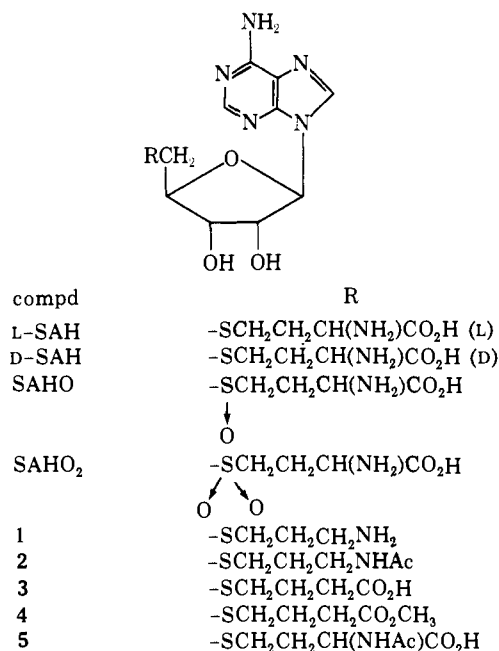
cently reported by Barchas and Deguchi,³ who observed that L-SAH is a potent inhibitor of several methyltransferases and that a stimulating factor for these enzymes in the rat brain is adenosylhomocysteinase, which degrades L-SAH.

L-SAH has been reported to inhibit the activity of many methylating enzymes, such as catechol O-methyltransferase (COMT),³⁻⁵ phenylethanolamine N-methyltransferase (PNMT),³ histamine N-methyltransferase (HMT),^{6,7} hydroxyindole O-methyltransferase (HIOMT),^{3,6} tRNA methyltransferase,⁸⁻¹⁰ S-adenosylmethionine-glycine N-methyltransferase⁸ and indolethylamine N-methyltransferase.¹¹ Various structural modifications of L-SAH have been carried out in an attempt to elucidate the specificity of the enzymatic binding site for SAH and to develop potential inhibitors of COMT^{5,12,13} and tRNA methyltransferase.^{9,10,14,15} In an attempt to detect differences in the

†Abbreviations used are: SAM, S-adenosyl-L-methionine; L-SAH, S-adenosyl-L-homocysteine; D-SAH, S-adenosyl-D-homocysteine; SAHO, S-adenosyl-L-homocysteine sulfoxide; SAHO₂, S-adenosyl-L-homocysteine sulfone; COMT, catechol O-methyltransferase (E.C. 2.1.1.6); HMT, histamine N-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole O-methyltransferase (E.C. 2.1.1.4); PNMT, phenylethanolamine N-methyltransferase (E.C. 2.1.1.1); K_s, inhibition constant for the slope.

binding specificity for L-SAH on various methyltransferases, we have synthesized a series of compounds with modifications in the amino acid portion of the SAH molecule (Chart I). Using these compounds as probes we have investigated the specificity of the L-SAH binding site on COMT, PNMT, HMT, and HIOMT. The present paper reports the results of this investigation.

Chart I. Compounds Synthesized to Probe the SAH Binding Sites of COMT, PNMT, HMT, and HIOMT



Experimental Section

Melting points were obtained on a calibrated Thomas-Hoover Uni-Melt and are corrected. Microanalyses were conducted on an F and M Model 185 C, H, N analyzer, The University of Kansas, Lawrence, Kan. Unless otherwise stated, the ir, nmr, and uv data were consistent with the assigned structures. Ir data were recorded on a Beckman IR-33 spectrophotometer, nmr data on a Varian Associates Model T-60 spectrophotometer (TMS), and uv data on a Cary Model 14 spectrophotometer. Scintillation counting was done on a Beckman LS-150 scintillation counter. Tlc were run on Analtech silica gel GF (250 μ) or Avicel F (250 μ). Spots were detected by visual examination under uv light and/or ninhydrin for compounds containing amino moieties.

Materials. SAM-¹⁴CH₃ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μ Ci/ml and stored at -20°F. SAM iodide (Sigma) was stored as a 0.01 M aqueous stock solution. N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES, Sigma) buffer, pH 7.60, was prepared as a 0.2 M stock solution. Phosphate buffers were prepared as 0.5 M stock solutions.

The following compounds were commercially available from the indicated sources: 3,4-dihydroxybenzoate (Aldrich); DL- β -phenylethanolamine, histamine dihydrochloride, N-acetylserotonin, L-SAH (Sigma). D-SAH was a gift from Dr. J. H. Hildesheim of the Institut de Chemie des Substances Naturelles, C.N.R.S., Gif-sur-Yvette, France. 5'-S-Adenosyl-L-N-acetylhomocysteine (5) was prepared from L-SAH and Ac₂O by the method of Hildesheim, *et al.*¹⁴

S-Adenosyl-L-homocysteine Sulfoxide (SAHO). SAHO was prepared by a modified literature procedure.¹⁶ S-Adenosyl-L-homocysteine (129 mg, 0.33 mmol) was suspended in glacial acetic acid (1 ml). To the slurry was added 30% hydrogen peroxide (0.12 ml, 1 mmol) with stirring at 20°, after which the reaction mixture was allowed to stand at ambient temperature for 16 hr. The reaction mixture was filtered and to the filtrate was added EtOH until solid formation was observed. The white solid which formed was collected by filtration and recrystallized (H₂O) to yield 91 mg (62%), mp 185-188° dec (lit.¹⁷ >100°). *Anal.* (C₁₄H₂₀N₆O₆S₃·3H₂O) C, H, N.

S-Adenosyl-L-homocysteine Sulfone (SAHO₂). SAHO₂ was

prepared by a modified literature procedure previously used to make DL-methionine sulfone and DL-ethionine sulfone.¹⁸ To 3 ml of H₂O was added 100 mg of ammonium molybdate and 0.7 ml of 70% perchloric acid. The mixture was heated to boiling for 5 min and the undissolved material was removed by filtration. The filtrate (0.5 ml) was cooled (0.5°) and L-SAH (96 mg, 0.25 mmol) was added. To the cooled slurry was added 30% hydrogen peroxide (2 ml) after which the reaction mixture was allowed to stand at ambient temperature for 10 hr. The reaction mixture was cooled (0-5°) and neutralized to pH 5 with *n*-butylamine, and EtOH was added until a precipitate formed. The white solid which formed was collected by filtration and recrystallized (H₂O-EtOH) to yield 50 mg (49%), mp 198° dec. *Anal.* (C₁₄H₂₀N₆O₇·S·H₂O) C, H, N.

5'-S-Adenosyl-3-thiopropylamine Bisulfate (1). 5'-S-Adenosyl-3-thiopropylamine (1) was prepared by the condensation of 2',3'-*O*-isopropylidene-5'-*O*-toluene-*p*-sulfonyl-adenosine (8, 1.85 g, 4 mmol) and 3-benzylthiopropylamine hydrochloride¹⁹ (870 mg, 4 mmol) in Na and liquid NH₃ according to the procedure of Jamieson.¹⁹ Treatment of the intermediate 2',3'-isopropylidene-5'-S-adenosyl-3-thiopropylamine (300 mg, 0.78 mmol) with 1 N H₂SO₄ for 48 hr at ambient temperature afforded 242 mg (90%) of the desired 5'-S-adenosyl-3-thiopropylamine bisulfate (1), mp 184-187° dec (lit.¹⁹ mp 180-183°).

5'-S-Adenosyl-3-N-acetylthiopropylamine (2). 2',3'-Isopropylidene-5'-S-adenosyl-3-thiopropylamine (510 mg, 1.4 mmol) was dissolved in a mixture of CH₃OH (16 ml) and Ac₂O (8 ml, 2.65 mmol). The reaction mixture was stirred at 0° for 10 hr after which time the solvent was removed under reduced pressure and the residue crystallized (H₂O) to yield 440 mg (81%) of 2',3'-isopropylidene-5'-S-adenosyl-3-N-acetylthiopropylamine; mp ca. 45° (very hygroscopic); nmr (CDCl₃) δ 1.40, 1.57 (2 s, 6 H, acetamide), 1.95 (s, 3 H, CH₃CO), 2.32-2.71 (m, 2 H, -CH₂CH₂S-), 2.82 (d, 2 H, 5'-CH₂), 3.00-3.50 (m, 2 H, -NHCH₂-); ir (KBr) 1700 cm⁻¹ (C=O).

2',3'-Isopropylidene-5'-S-adenosyl-3-N-acetylthiopropylamine (480 mg, 1.18 mmol) was dissolved in 10 ml of 0.5 N H₂SO₄ and stirred at ambient temperature for 24 hr. The reaction mixture was diluted with 15 ml of H₂O and neutralized with lead carbonate to pH 5.0. The suspension was filtered and the filtrate was lyophilized. The product was recrystallized (H₂O) to yield 456 mg (80%) of 2, mp 68-70°. *Anal.* (C₁₅H₂₂N₆O₄S) C, H, N.

S-Benzyl-4-thioethylmalonic Acid (6). S-Benzyl-4-thioethylmalonic acid (6) was prepared by a modification of the procedure of du Vigneaud and Brossworth.²⁰ The intermediate diethyl S-benzyl-4-thioethylmalonate was prepared by reaction of sodium methoxide (2.2 g), diethyl malonate (9.6 g, 60 mmol), and benzyl- β -chloroethyl sulfide²⁰ (7.46 g, 40 mmol) in absolute EtOH. The pure diethyl S-benzyl-4-thioethylmalonate was isolated by distillation to yield a colorless oil; 6.5 g (52%); bp 144-146° (0.07 mm).

Diethyl S-benzyl-4-thioethylmalonate (6.0 g, 20 mmol) was hydrolyzed to 6 by using 12.5% NaOH in 50% aqueous EtOH and refluxing for 2 hr. The EtOH was removed by distillation, and as the volume decreased H₂O was added so that the final volume was about 120 ml. The reaction mixture was cooled and 40 ml of concentrated HCl was slowly added. The precipitate which formed was filtered with H₂O resulting in the isolation of 5.4 g (92%) of 6, mp 117-119° (lit.²⁰ mp 114-117°). The product could be recrystallized (C₆H₆-Et₂O) to yield an analytical sample, mp 120-121°.

S-Benzyl-4-thiobutyric Acid (7). S-Benzyl-4-thiobutyric acid (7) was prepared by heating S-benzyl-4-thioethylmalonic acid (5 g, 20 mmol) in a round-bottom flask at 142° for 2 hr. The reaction mixture was cooled and the residue extracted with CHCl₃. Removal of the CHCl₃ under reduced pressure and distillation of the product afforded 4.1 g (98%) of 7; bp 150-152° (0.14 mm); nmr (CDCl₃) δ 1.86 (m, 2 H, -SCH₂CH₂-), 2.4 (t, 4 H, -SCH₂-CH₂CH₂-), 3.63 (s, 2 H, C₆H₅CH₂S-), 7.23 (s, 5 H, aromatic). This material was pure enough to be utilized for subsequent steps.

2',3'-Isopropylidene-5'-S-adenosyl-4-thiobutyric Acid (9). S-Benzyl-4-thiobutyric acid (7, 1.1 g, 5.2 mmol) was dissolved in 50 ml of liquid NH₃ which was surrounded by a Dry Ice-acetone bath and Na was added in small portions until a deep blue color persisted for 15 min. 2',3'-*O*-Isopropylidene-5'-*O*-toluene-*p*-sulfonyl-adenosine (8) (3.1 g, 6.7 mmol) was added and the reaction mixture was stirred and maintained at Dry Ice-acetone temperatures for 16 hr after which time the NH₃ was slowly allowed to evaporate. The solid residue was dissolved in 30 ml of H₂O and filtered. The filtrate (pH ~8.0) was washed several times with CHCl₃ and then adjusted to pH 5.0 using 5% HCl. The solid

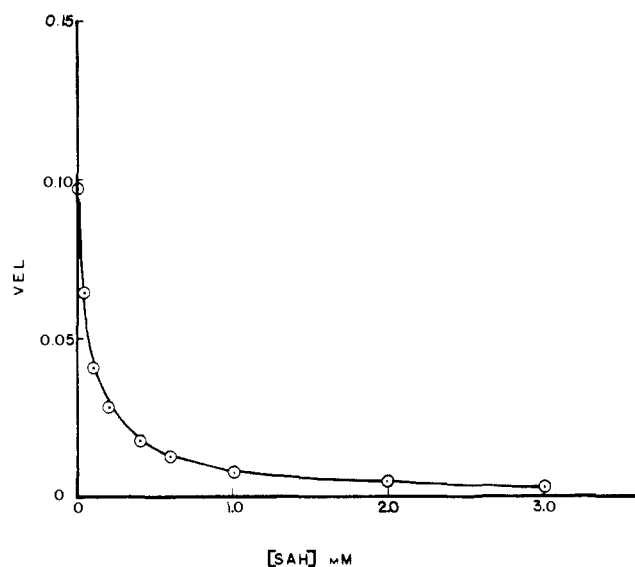


Figure 1. Inhibition of HIOMT activity with L-SAH. Assay conditions outlined in the Experimental Section except SAM concentration, 1 mM. Vel = nmol of product/mg of protein/min.

which formed was collected by filtration, washed several times with H₂O, and recrystallized (CHCl₃-hexane) to yield 1.76 g (83%), mp 158.5–160.5°. An analytical sample was prepared by recrystallization (CHCl₃-hexane), which afforded pure 9, mp 160–161°. Anal. (C₁₇H₂₃N₅O₅S) C, H, N.

5'-S-Adenosyl-4-thiobutyric Acid (3). 2',3'-Isopropylidene-5'-S-adenosyl-4-thiobutyric acid (1 g, 2.5 mmol) was dissolved in 20 ml of 0.5 N H₂SO₄ and stirred at ambient temperature for 34 hr. The reaction mixture was then adjusted to pH 5.0 using concentrated NaOH. The precipitate which formed was filtered, washed several times with H₂O, and recrystallized (CH₃OH) to yield 782 mg (87%) of 3, mp 205–208°. Anal. (C₁₄H₁₉O₅N₅S) C, H, N.

5'-S-Adenosyl-4-thiobutyric Acid Methyl Ester (4). To a suspension of 3 (360 mg, 1.0 mmol) in 50 ml of MeOH at 0–5° was added an excess of an ethereal solution of diazomethane [generated by slowly adding *N*-nitrosomethylurea (5 g) to a mixture of 50 ml of H₂O and 20 ml of 50% KOH at –10°]. The reaction mixture was maintained at 0–5° for 3 hr after which time the excess diazomethane was destroyed by dropwise addition of acetic acid. The solvent was removed under reduced pressure and the residue dissolved in 2 ml of CHCl₃ and applied to a silica gel plate (1000 μ, Analtech). The tlc was eluted with 10% EtOH-CHCl₃ and the material with an *R_f* of 0.15 was removed from the silica gel by extraction with CH₃OH. The CH₃OH was removed under reduced pressure, the residue was dissolved in 20 ml of CHCl₃ and filtered, and the CHCl₃ was removed under reduced pressure. The residue was dissolved in 10 ml of hot H₂O and cooled and the white solid which formed was collected by filtration to yield 125 mg (33%) of 4; mp 53–54°; nmr (acetone-*d*₆) δ 3.52 (s, 3 H, –OCH₃). Anal. (C₁₅H₂₁O₅N₅S·H₂O) C, H, N.

Enzyme Purification and Assay. COMT was purified from rat liver (male, Sprague-Dawley, 180–200 g) according to the methods previously described.^{21,22} The enzyme was purified through the calcium phosphate gel step resulting in a preparation which contained 3.84 mg of protein per milliliter. COMT was assayed using dihydroxybenzoate (2.0 mmol) as a substrate according to a previously described radiochemical procedure.²²

PNMT was purified from bovine adrenal medulla (Pel-Freez Biologicals) according to the procedure of Connert and Kirshner.²³ The purification was carried through the isoelectric precipitation and dialysis step, which resulted in a sixfold purification of the enzyme, which contained 8.0 mg of protein per milliliter. PNMT was assayed using a previously described radiochemical assay²³ and a normal incubation mixture consisted of the following components (in μmol): water, so that the final volume was 0.25 ml; DL-β-phenylethanolamine (0.25); S-adenosyl-L-methionine (variable); inhibitor (variable); 0.05 μCi of S-adenosyl-L-methionine-*methyl*-¹⁴C; phosphate buffer, pH 8.0 (25); and the enzyme preparation (0.050 ml). Incubation was carried out for 30 min at 37°, the reaction stopped with 0.25 ml of 0.5 M borate buffer (pH 10), and a mixture of toluene-isoamyl alcohol (7:3) used to extract the product.²³

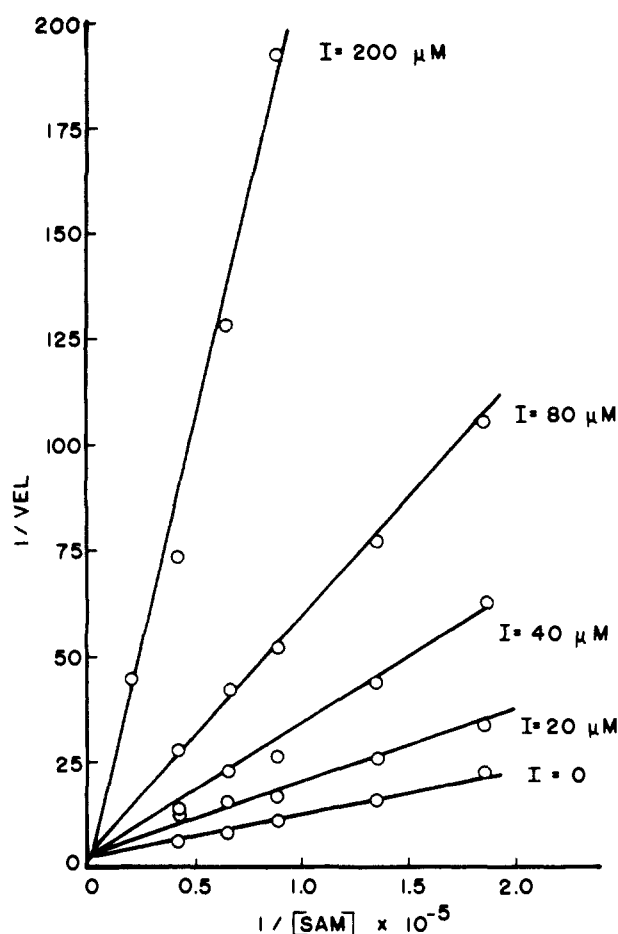


Figure 2. L-SAH inhibition of HIOMT. Reciprocal plots with SAM as the variable substrate. Assay conditions outlined in the Experimental Section except SAM concentration, 5.4–23.4 μM. *N*-Acetylserotonin concentration, 1.0 mM. Vel = nmol of product/mg of protein/min.

HMT was purified from guinea pig brain (Pel-Freez Biologicals) according to the methods previously described by Brown, *et al.*²⁴ The enzyme was purified through the dialysis step resulting in a preparation which contained 15.2 mg of protein per milliliter. This represented a ninefold purification from the crude supernatant. Kinetic experiments were carried out with an incubation mixture containing the following components (in μmol) added in this sequence: water, so that final volume was 0.25 ml; histamine (0.5); inhibitor (variable); S-adenosyl-L-methionine (variable); 0.05 μCi of S-adenosyl-L-methionine-*methyl*-¹⁴C; phosphate buffer, pH 7.40 (10); and the enzyme preparation (0.05 ml). Incubation was carried out for 30 min at 37°. The reaction was stopped by addition of 0.25 ml of 0.5 M borate buffer (pH 10.0) and the aqueous mixture extracted with 10 ml of a toluene-isoamyl alcohol (1:1). After centrifugation, an aliquot (5 ml) of the organic phase was transferred to a scintillation vial, and a dioxane-based phosphor solution (10 ml) was added and the radioactivity measured.

HIOMT was isolated from bovine pineal glands (Pel-Freez Biologicals) and purified through the DEAE-Sephadex chromatography step according to the procedure of Jackson and Lovenberg.²⁵ This afforded a preparation which contained 20.5 mg of protein per milliliter which represented a tenfold purification from the crude supernatant. Assay of HIOMT was carried out using a previously described assay²⁶ with a normal incubation mixture containing (in μmol): water, so that the final volume was 0.30 ml; *N*-acetylserotonin (0.25); inhibitor (variable); S-adenosyl-L-methionine (variable); 0.05 μCi of S-adenosyl-L-methionine-*methyl*-¹⁴C; phosphate buffer, pH 7.90 (10); and the enzyme preparation (0.05 ml). Incubation was carried out for 60 min at 37°, the reaction was stopped by addition of a 0.10 ml of 0.5 M borate buffer (pH 10.0), and the radioactive product was extracted with 10 ml of toluene-isoamyl alcohol (5:1).²⁶

Data Processing. Reciprocal velocities were plotted graphically against reciprocals of the substrate concentrations. In all cases

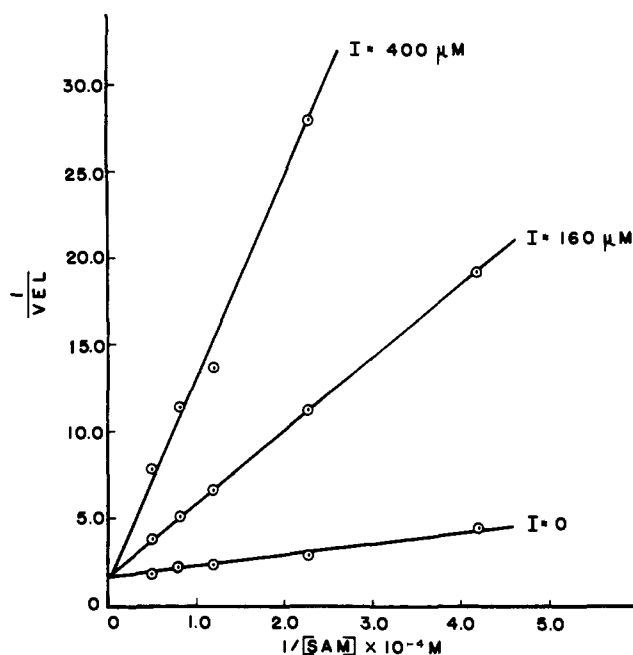


Figure 3. L-SAH inhibition of PNMT. Reciprocal plots with SAM as the variable substrate. Assay conditions outlined in the Experimental Section except SAM concentration, 24–210 μM . DL- β -Phenylethanolamine concentration, 1.0 mM. Vel = nmol of product/mg of protein/min.

reasonably linear relationships were obtained. These data were then fitted to the appropriate equations and the inhibition constants calculated using a Hewlett-Packard 2100 A digital computer and a Fortran IV program.^{22,27,28}

Results and Discussion

Chemistry. The analogs of SAH synthesized for this study are listed in Chart I. These derivatives and their synthetic intermediates were characterized by several methods, including their spectral and chromatographic properties and elemental analyses. The general route for the synthesis of SAH analogs is the reaction of S-benzylhomocysteine or a derivative of S-benzylhomocysteine in Na-liquid NH_3 with the requisite halide or tosylate.²⁹ For example, the deaminated SAH **3** was prepared by the condensation of S-benzyl-4-thiobutyric acid (**7**) and the tosylate **8** in Na-liquid NH_3 , which afforded the intermediate isopropylidene derivative **9**. S-Benzyl-4-thiobutyric acid (**7**) was prepared by decarboxylation of **6**.²⁰ Hydrolysis of **9** in 0.5 N H_2SO_4 afforded the desired 5'-S-adenosyl-4-thiobutyric acid (**3**). Treatment of **3** with diazomethane afforded the corresponding methyl ester **4** (Scheme I).

Enzyme Inhibitory Activity. L-SAH has previously been reported to inhibit the activity of many methylating enzymes, including COMT,³⁻⁵ PNMT,³ HMT,^{6,7} and HIOMT.^{3,6} The effect of added L-SAH on the initial velocity of the HIOMT-catalyzed transmethylation reaction is shown in Figure 1. Under the experimental conditions used, 50% inhibition of HIOMT activity was obtained with 50 μM L-SAH. The kinetics of SAH inhibition with varying SAM have previously been shown to be competitive with the enzymes COMT³⁰ and HMT.⁷ Using reciprocal velocity vs. reciprocal SAM plots, the kinetic patterns for inhibition of PNMT and HIOMT by L-SAH were determined and the results are shown in Figures 2 and 3. In both cases a linear competitive pattern of inhibition was observed when SAM was the variable substrate.

The various structural analogs of SAH, which were synthesized as part of this study, were tested as inhibitors of COMT, PNMT, HMT, and HIOMT. The results are shown in Table I. For each methyltransferase studied

Table I. Inhibition of COMT, PNMT, HMT, and HIOMT by SAH Analogs^a

Compd	Inhibitor ^b concn, mM	% inhibition			
		COMT	PNMT	HMT	HIOMT
L-SAH	0.2	39	49	40	71
	2.0	87	92	89	94
D-SAH	0.2	5	14	73	1
	2.0	22	32	99	6
SAHO	0.2	14	10	0	0
	2.0	42	22	9	0
SAHO ₂	0.2	8	13	0	0
	2.0	45	17	10	0
1	0.2	1	5	17	0
	2.0	6	11	33	0
2	0.2	2	4	0	0
	2.0	7	10	15	0
3	0.2	4	6	0	0
	2.0	8	11	0	0
4	0.2	0	0	0	0
	2.0	0	0	0	0
5	0.2	1	7	0	0
	2.0	10	10	0	0

^aCOMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section except in each case the SAM concentration = 1.0 mM. ^bThe inhibitors were prepared in aqueous stock solutions (10.0 $\mu\text{mol/ml}$).

strong inhibition by L-SAH was observed. The enzyme showing the highest specificity for the structural features of L-SAH was HIOMT, since none of the analogs tested showed appreciable inhibitory activity on this enzyme. Relative to L-SAH, D-SAH showed a substantial reduction in inhibitory activity on COMT, PNMT, and HIOMT. However, in contrast, D-SAH showed higher inhibitory activity than L-SAH with HMT. This latter observation would indicate drastic differences in the configurational requirements associated with the SAH binding site on HMT, as compared to the sites on COMT, PNMT, and HIOMT. D-SAH had previously been shown to be a potent inhibitor of *Escherichia coli* tRNA methyltransferase.¹⁰

Modifications of the sulfur atom on the homocysteine portion of SAH result in a reduction in inhibitory activity. With the enzymes COMT and PNMT, SAHO and SAHO₂ show significant inhibitory activity; however, with HMT and HIOMT these analogs of SAH are essentially inactive. This would indicate that with HMT and HIOMT the

Scheme I

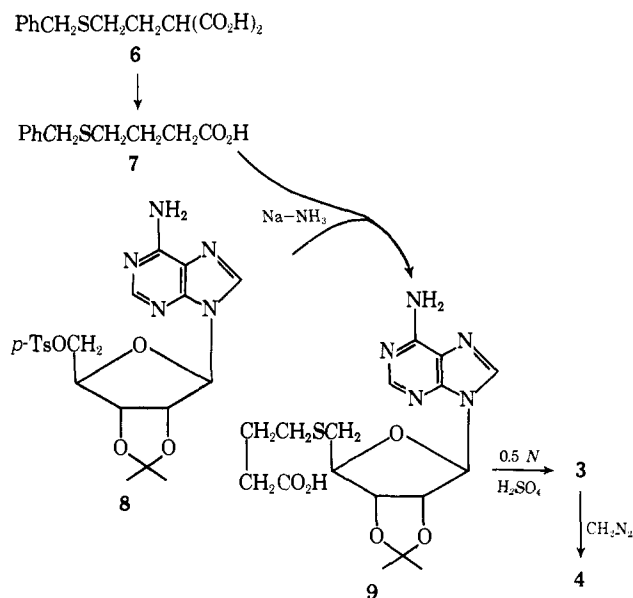


Table II. Inhibition Constants for Various SAH Analogs toward COMT, PNMT, HNMT, and HIOMT^a

Inhibitor	Inhibition constants, μM , ^{b,c} $K_{is} \pm S.E.M.$			
	COMT	PNMT	HMT	HIOMT
L-SAH	36.3 \pm 2.20	29.0 \pm 2.84	18.1 \pm 2.19	18.5 \pm 1.9
D-SAH	1611 \pm 120	623 \pm 45	10.5 \pm 1.3	
SAHO	482 \pm 47	2372 \pm 463		
SAHO ₂	894 \pm 47	3486 \pm 407		
1			1482 \pm 178	

^aCOMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section except in each case SAM concentrations, 24–210 μM . ^bEach inhibitor showed linear competitive kinetics and the inhibition constants were calculated as previously described.²² ^cWhen low inhibitory activity was observed from the preliminary studies, no extensive studies were done to determine the kinetic inhibition constants.

binding through the sulfur atom of SAH may be more important than is the case for COMT and PNMT. The results obtained for SAHO inhibition of COMT are similar to those observed by Coward, *et al.*,⁵ who had previously reported that SAHO was *ca.* $\frac{1}{20}$ as effective as SAH as an inhibitor of COMT.

The terminal amino group of L-SAH appears to be essential for activity, since *N*-acetyl SAH 5 and the deaminated SAH derivatives 3 and 4 all showed little or no inhibitory activity on the methyltransferases tested. The importance of the terminal carboxyl group in the binding of SAH is indicated by the reduced inhibitory activity of the decarboxylated SAH derivatives 1 and 2. With PNMT, COMT, and HIOMT the decarboxylated SAH 1 showed little or no inhibitory activity; however, with HMT some inhibitory activity of 1 was still observed. The inhibitory activity of 1 and D-SAH with HMT indicates that at the binding site of this enzyme, the carboxyl group of SAH may contribute less to the overall binding than would be the situation at the binding site of COMT, PNMT, and HIOMT, where having both the amino and carboxyl groups in the proper orientation is an absolute requirement for maximum activity. However, at the binding site on HMT, the terminal carboxyl group of L-SAH must still be an important point of attachment because 1 is substantially less active than L-SAH or D-SAH.

Using reciprocal velocity *vs.* reciprocal SAM plots, the kinetic patterns for inhibition of COMT, PNMT, HMT, and HIOMT by the active SAH analogs (L-SAH, D-SAH, SAHO, SAHO₂, and 1) were determined and the resulting inhibition constants are given in Table II. In all cases linear competitive patterns of inhibition were observed when SAM was the variable substrate. For example, in Figure 4 is shown the kinetic pattern for inhibition of HMT by D-SAH. These competitive kinetic patterns support, but do not prove, the identity of the binding sites for SAH and for its various structural analogs. The large differences in K_{is} values suggest that in making these rather minor structural changes, some of the interactions critical to formation of the E-I complex have been grossly disturbed. The exception appears to be the inhibitory effects produced by D-SAH on HMT, where perhaps the configuration of the amino and carboxyl groups offered by D-SAH is more desirable for binding to the active site of HMT than is the orientation of those groups provided by L-SAH.

From the kinetic inhibition data of these structural analogs of SAH (Table II), it can be concluded that the binding site on HMT is better able to accommodate changes in the configuration of the amino acid asymmetric carbon of SAH. In contrast, the binding site on COMT appears better able to accommodate changes in the oxidation state of the sulfur atom of SAH, since the inhibition constants for SAHO and SAHO₂ are only 10–20 times greater than that for L-SAH. This can be compared to the data for PNMT where the inhibition constants for SAHO and SAHO₂ are 100–200 times greater than that for 1.

SAH. With HMT and HIOMT these oxidized derivatives were completely inactive.

Conclusions

The present paper has attempted to delineate the structural features of the homocysteine portion of L-SAH which are required for binding of SAH by COMT, PNMT, HMT, and HIOMT. The approach which was taken was to make minor changes in the amino acid portion of SAH but not to alter the base or sugar portions of this molecule. The modifications have involved changes in the configuration of the amino acid asymmetric carbon, derivatization and/or removal of the terminal amino and carboxyl groups, and changes in the oxidation state of the sulfur atom. In general it was concluded from this study that the terminal amino and terminal carboxyl groups present in the L configuration are an absolute requirement for the SAH molecule to bind with COMT, PNMT, and HIOMT.

From modifications of the sulfur atom of SAH, it was concluded that the sulfur atom is directly involved in binding, since changes in its oxidation state resulted in decreased affinity for the binding sites. An alternative explanation is that the area on the enzyme which accommodates the $-\text{CH}_2\text{SCH}_2-$ portion of SAH is sensitive to changes in the steric bulk of the ligand and that the decreased affinity of SAHO and SAHO₂ may be a result of steric rather than electronic effects.

The interesting exception to what appears to be high enzyme-ligand specificity was the inhibitory effect of D-SAH on HMT. With COMT, PNMT, and HIOMT, D-SAH was essentially inactive as an inhibitor; however, with HMT, D-SAH was a significantly better inhibitor than L-SAH. A $K_{is} = 10.5 \pm 1.3 \mu M$ was observed for D-SAH inhibition of HMT compared to a $K_{is} = 18.1 \pm 2.19 \mu M$ for L-SAH. From the other derivatives studied it was shown that both the terminal amino and carboxyl groups are required for binding of SAH to HMT. Therefore, it has been concluded that the configuration of these groups offered by D-SAH is more desirable for binding than the configuration offered by L-SAH to the HMT binding site. A possible explanation for this observation is shown in Chart II, where normal binding of the homocysteine portion of L-SAH to an enzyme is shown. The importance of the terminal amino group, terminal carboxyl group, and the sulfur atom for the activity of L-SAH, as shown in this study, indicates specific involvement of these functional groups in binding. Therefore, three points of attachment for this portion of L-SAH have been proposed (Chart II). The points of attachment shown are similar to those proposed earlier for the binding of SAM to various methyltransferases.⁶ For the binding of D-SAH to this site, it is only possible to bind the ligand to the enzyme through two of the three normal attachment sites on the enzyme surface, which in the case of COMT, PNMT, and HIOMT must result in very weak binding and account for the reduced inhibitory activity of D-SAH. The

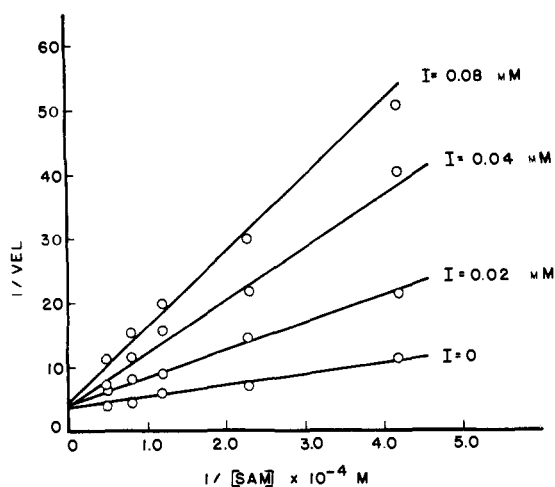
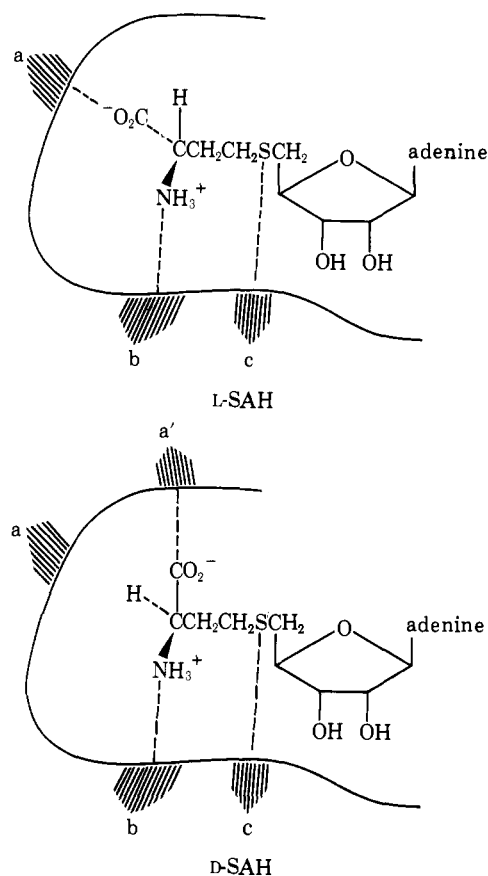


Figure 4. D-SAH inhibition of HMT. Reciprocal plots with SAM as the variable substrate. Assay conditions outlined in the Experimental Section except SAM concentration, 24–210 μM . Histamine concentration, 1.0 mM. Vel = nmol of product/mg of protein/min.

two points of attachment shown in Chart II are through the terminal amino group and sulfur atom. It is felt that the side chain of the amino acid, which contains the sulfur atom, as well as the sugar and base portion of the molecule must be maintained in the orientation shown, since binding at the 6-amino group of the adenine portion of SAH is also an absolute requirement for activity.³¹ The increased affinity of D-SAH for the HMT binding site is, therefore, explained in Chart II by the existence of an alternative binding site (a') for the carboxyl group of SAH.

Chart II. Proposed Enzymatic Binding Sites for L-SAH and D-SAH^a



^aAttention is given only to the binding of the homocysteine portion of SAH (see text for discussion).

In fact, the binding of the terminal carboxyl group of D-SAH by this alternative site produces a stronger interaction between D-SAH and HMT than that produced with L-SAH. A similar type of argument can be put forth for D-SAH binding through the normal sulfur atom and terminal carboxyl group attachment sites, with the existence of an alternative binding site for the terminal amino group of SAH.

In general, it can be concluded from this study that COMT, PNMT, HMT, and HIOMT show fairly high specificity for the structural features of the homocysteine portion of L-SAH. This high specificity for L-SAH appears to be common for most methyltransferases.^{5,6,9,10,12,13} However, some interesting and potentially useful differences in the binding sites of these methyltransferases have been uncovered in this study. These differences in the binding specificity are being further explored in an attempt to design specific inhibitors of these enzymes.

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Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 2. Modification of the Base Portion of *S*-Adenosylhomocysteine

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The specificity of *S*-adenosyl-L-homocysteine (SAH) inhibition of enzymatic transmethylation was explored by preparing structural analogs of SAH in which the base portion of the molecule was modified. The various SAH analogs have been evaluated as inhibitors of catechol *O*-methyltransferase, phenylethanolamine *N*-methyltransferase, histamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase. Inhibition studies indicated that there exists a specificity by these enzymes for the adenine portion of SAH, with an absolute requirement of the 6-amino group for maximum activity. Substitution of other pyrimidine and purine bases in place of adenine resulted in complete loss of activity. However, minor modifications of the adenine moiety of SAH could be tolerated at the enzymatic binding sites. For example, *S*-3-deazaadenosyl-L-homocysteine was found to have inhibitory activity similar to SAH itself. Some differences in the binding requirements of these methyltransferases have been observed so that differential inhibition may be possible.

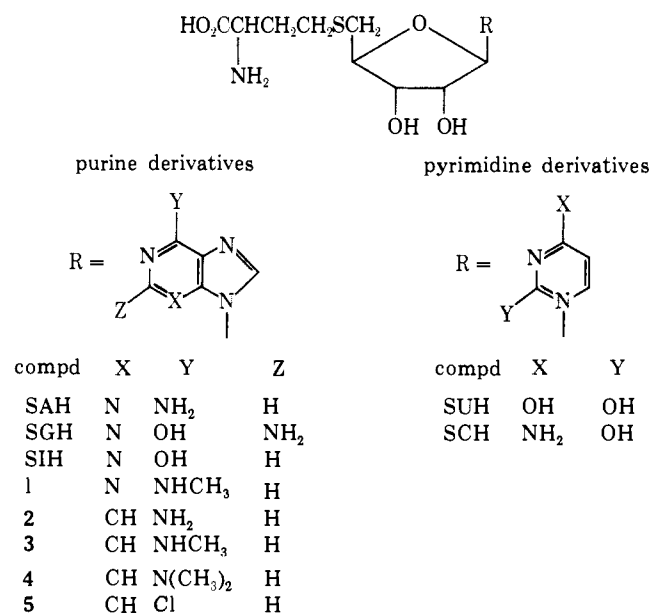
A general feature of most *S*-adenosylmethionine (SAM)[†] dependent methyltransferases is the inhibition produced by the demethylated product *S*-adenosyl-L-homocysteine (SAH). This product inhibition by SAH suggests a possible biological regulatory mechanism for transmethylation reactions,¹ as well as a new class of potential inhibitors of methyltransferases.²⁻¹⁰ In the preceding paper of this series,² we described the synthesis and enzymatic evaluation of a series of compounds with modification only in the homocysteine portion of the SAH molecule. It was concluded from this study that all of the functional groups of the homocysteine portion of SAH are required for maximum binding of SAH to these enzymes. Furthermore, it was proposed² that there exists three points of attachment of the homocysteine portion of SAH to the enzyme site. These points of attachment are the terminal amino group, the terminal carboxyl group, and the sulfur atom. In order to further study the nature of the intermolecular forces involved in the binding of SAH, we have synthesized a series of SAH derivatives with modification only in the base portion of the SAH molecule (Chart I). The methyltransferases of interest in this study were catechol *O*-methyltransferase (COMT), phenylethanolamine *N*-methyltransferase (PNMT), histamine *N*-methyltransferase (HMT), and hydroxyindole *O*-methyltransferase (HIOMT). Using the base-modified SAH analogs (Chart I) as probes of the active sites of COMT, PNMT, HMT, and HIOMT, we have delineated the contributions of the adenine moiety in the enzymatic binding of SAH. The present paper reports the results of this investigation.

Experimental Section

The general experimental techniques and equipment used in this study were described in detail in the preceding paper in this

[†]Abbreviations used are: SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine; SGH, *S*-guanosyl-L-homocysteine; SIH, *S*-inosyl-L-homocysteine; SCH, *S*-cytidyl-L-homocysteine; SUH, *S*-uridyl-L-homocysteine; *N*⁶-methyl-SAH, *S*-*N*⁶-methyladenosyl-L-homocysteine; 3-deaza-SAH, *S*-3-deazaadenosyl-L-homocysteine; *N*⁶-methyl-3-deaza-SAH, *N*⁶-methyl-3-deazaadenosyl-L-homocysteine; *N*⁶-dimethyl-3-deaza-SAH, *N*⁶-dimethyl-3-deazaadenosyl-L-homocysteine; COMT, catechol *O*-methyltransferase (E.C. 2.1.1.6); PNMT, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1.1); HMT, histamine *N*-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole *O*-methyltransferase (E.C. 2.1.1.4); *K*_{1/2}, inhibition constant for the slope; *K*₁₁, inhibition constant for the intercept.

Chart I. Derivatives of SAH Synthesized to Probe the Binding Sites of COMT, PNMT, HMT, and HIOMT



series.² The nmr assignments for the 3-deazaadenosine derivatives are given in terms of the purine numbering system, rather than the imidazo[4,5-c]pyridine numbering system.

The following compounds were commercially available from the indicated sources: 3,4-dihydroxybenzoate (Aldrich); DL-β-phenylethanolamine, histamine dihydrochloride, *N*-acetylserotonin, SAH, 2',3'-isopropylideneuridine, 2',3'-isopropylideneuridine, 2',3'-isopropylideneinosine, 2',3'-isopropylideneadenosine, and *S*-benzyl-L-homocysteine (Sigma). *N*⁶-Methyl-SAH (1) was a gift from Dr. J. Hildesheim of the Institut de Chimie des Substances Naturelles, C.N.R.S., Gif-sur-Yvette, France. SUH and SGH were prepared by Hildesheim, *et al.*^{6,7}

***S*-Inosyl-L-homocysteine (SIH).** 2',3'-Isopropylideneinosine (2.0 g, 6.2 mmol) was dissolved in 20 ml of dry pyridine, freshly distilled from barium oxide, and the resulting solution was cooled to ca. 0–5° in an ice-salt bath. Freshly recrystallized *p*-toluenesulfonyl chloride (1.30 g, 6.8 mmol) was added to the cooled solution in one portion and the reaction mixture was stirred for 12 hr at ambient temperature. The pyridine was removed under reduced pressure and H₂O was added to the residue. The resulting aqueous solution was extracted several times with CHCl₃. The CHCl₃ layer was then washed with H₂O and 5% NaHCO₃ and