Potential Inhibitors of S-Adenosylmethionine-Dependent Methyltransferases. 6. Structural Modifications of S-Adenosylmethionine

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Structural analogues of S-adenosyl-L-methionine (SAM), with modifications in the amino acid, sugar, or base portions of the molecule, have been synthesized and evaluated as either inhibitors and/or substrates for the enzymes catechol O-methyltransferase, phenylethanolamine N-methyltransferase, histamine N-methyltransferase, and hydroxyindole O-methyltransferase. To evaluate these analogues as substrates for SAM-dependent methyltransferases, the corresponding methyl-^{14}C compounds were prepared and tested for their abilities to donate their methyl group to the appropriate acceptor molecules. In addition, the unlabeled SAM analogues were tested for their inhibitory activities in these same transmethylation reactions. In general, it could be concluded from these studies that methyltransferases show very strict specificity for the structural features of SAM. This strict specificity holds for the enzymatic binding and methyl-donating abilities of this molecule. In fact, it could be concluded from the results of this study that methyltransferases show a higher specificity for the structural features of the substrate L-SAM than for the structural features of the product S-adenosyl-L-homocysteine (L-SAH).

S-Adenosyl-L-methionine (L-SAM)[2] has been shown to be the natural methyl donor in many enzyme-catalyzed transmethylation reactions.[3] Structural modifications of this biological methyl donor have to date been fairly limited in nature and scope; however, some modifications of the sulfonium pole of L-SAM have been reported.[4-7] For example, the sulfur atom has been replaced by selenium,[4a,b] and the methyl group of L-SAM has been replaced by an ethyl group (S-adenosyl-L-ethionine)[4b,c] or by an n-propyl group [S-adenosyl(S-n-propyl)-L-homocysteine].[5] In addition, the chemical synthesis of (±)-L-SAM has permitted the elucidation of the stereochemical requirements of the sulfonium pole for the enzymatic binding and methyl-donating abilities of this molecule.[6,7]

Structural analogues of L-SAM with modifications in the amino acid or base portions have been prepared in very limited number. Zappia et al.[8] have reported the synthesis and methyl donor activity of four structural analogues of L-SAM including S-inosyl-L-methionine (SIM), S-adenosyl-3-methylthiopropylamine (decarboxylated SAM), S-inosyl-L-(2-hydroxy-4-methylthio)butyric acid, and S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid. In addition, Montgomery et al.[9] have reported the synthesis of various analogues of 5′-deoxy-5′-(methylthio)adenosine.

Because of the limited number of analogues of L-SAM having been synthesized, the structural features of L-SAM which are involved in enzymatic binding and are required for the methyl-donating abilities of this molecule remain unknown. By synthesizing an extensive series of analogues of S-adenosyl-L-homocysteine (L-SAH) and testing their inhibitory activities toward specific methyltransferases, our laboratory[10-15] has previously elucidated some of the structural features of L-SAH which are required for its enzymatic binding. In a similar study we report here the chemical synthesis of a series of L-SAM analogues from the corresponding L-SAH compounds in which structural modifications have been made in the amino acid, sugar, or base portions of the SAM molecule (Charts I and II). We also report here the activity of these SAM analogues as inhibitors of transmethylation reactions as well as the activity of the isotopically labeled ($^{14}CH_3$) SAM analogues as substrates for methyltransferases. The enzymes of interest in this study were catechol O-methyltransferase (COMT), phenylethanolamine N-methyltransferase (PNMT), histamine N-methyltransferase (HMT), and hydroxyindole O-methyltransferase (HIOMT).

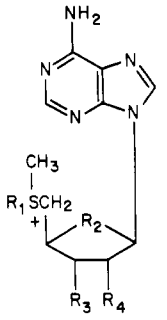
Experimental Section

The general experimental techniques and equipment used in this study were described in detail in the preceding papers in this series.[7,14] The following compounds are commercially available from the indicated sources: 3,4-dihydroxybenzoate (Aldrich); DL-β-phenylethanolamine, histamine dihydrochloride, N-acetylserotonin, SAM iodide (Sigma); SAM-$^{14}CH_3$ (55.0 mCi/mmol, New England Nuclear); $^{14}CH_3I$ (55.0 mCi/mmol, Amersham/Searle).

Each of the SAM analogues reported here (Charts I and II) was prepared by reaction of the corresponding SAH analogues with methyl iodide according to a modification of the procedure of Jamieson.[16] The SAH analogues used were synthesized according to previously published procedures as cited below: S-adenosyl-D-homocysteine,[17] S-adenosyl-L-cysteine,[14,18] S-adenosyl-3-thiopropylamine,[10] S-adenosyl-4-thiobutyric acid,[10] S-adenosyl-L-N-acetylhomocysteine,[10] S-2′-deoxyadenosyl-L-homocysteine,[12,17] S-3′-deoxyadenosyl-L-homocysteine,[12,17] S-aristeromycinyl-L-homocysteine,[13] S-inosyl-L-homocysteine,[11] S-guanosyl-L-homocysteine,[11] S-tubercidinyl-L-homocysteine,[14,17] S-8-azaadenosyl-L-homocysteine,[14] N^6-methyladenosyl-L-homocysteine,[11,17] N^6-dimethyladenosyl-L-homocysteine,[14] 3-deazaadenosyl-L-homocysteine,[11] N^6-methyl-3-deazaadenosyl-L-homocysteine,[11,17] N^6-dimethyl-3-deazaadenosyl-L-homocysteine,[11] S-uridyl-L-homocysteine,[11] and S-cytidyl-L-homocysteine.[11]

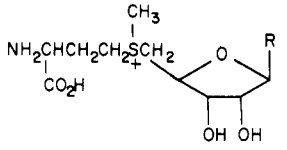
General Procedure for the Conversion of SAH Analogues to the Corresponding Unlabeled SAM Analogues. The general procedure previously described by Jameison[16] was used with modifications. A structural analogue of SAH (50 mg) was dissolved in formic acid (2 ml) or a mixture of formic acid (1 ml) and acetic acid (1 ml). Methyl iodide (0.5 ml, 8 mmol) was added to the solution, which was shaken vigorously and then kept in the dark at room temperature for about 5 days. Ice-cold water (ca. 5 ml) was then added to the reaction mixture and the unreacted methyl iodide was extracted with ice-cold ether. The aqueous layer was lyophilized, and the resulting powder was dissolved immediately in pH 7.0, 0.01 M phosphate buffer. The phosphate buffer solution containing the desired sulfonium compound was then applied to a column (2 × 8 cm) of Amberlite IRC-50 ion-exchange resin previously equilibrated with 0.01 M phosphate buffer, pH 7.0. The unreacted thioether (SAH analogue) was eluted with 100 ml of 0.01 M phosphate buffer, pH 7.0, and an intermediate fraction was eluted with 50 ml of 0.25 N acetic acid. The sulfonium nucleoside was then eluted with about 50 ml of 4 N acetic acid and the resulting eluate was lyophilized to yield a white powder. The product was immediately dissolved in water and the concentration of the sulfonium nucleoside determined by the uv absorbance using as reference the extinction coefficient of the corresponding SAH analogue. Yields of the SAM analogues were generally 75–90%. These aqueous samples were immediately used for the enzyme experiments or stored at −10 °C. The new SAM analogues were not obtained in crystalline form, since the instability of such nucleoside sulfonium compounds prohibited crystallization by classical techniques. The new SAM analogues were characterized by their chromatographic properties (see Table I for chromatographic systems used and the characteristic R_f values), their spectral

Chart I. Analogues of SAM with Modifications in the Amino Acid or Sugar Portions



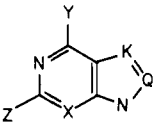
Compd	R_1	R_2	R_3	R_4
L-SAM	$-CH_2CH_2CH(NH_2)CO_2H$ (L)	O	OH	OH
D-SAM	$-CH_2CH_2CH(NH_2)CO_2H$ (D)	O	OH	OH
S-Me-SAC	$-CH_2CH(NH_2)CO_2H$ (L)	O	OH	OH
decarboxylated SAM	$-CH_2CH_2CH_2NH_2$	O	OH	OH
deaminated SAM	$-CH_2CH_2CH_2CO_2H$	O	OH	OH
N^{α}-Ac-SAM	$-CH_2CH_2CH(NHAc)CO_2H$ (L)	O	OH	OH
2′-deoxy-SAM	$-CH_2CH_2CH(NH_2)CO_2H$ (L)	O	OH	H
3′-deoxy-SAM	$-CH_2CH_2CH(NH_2)CO_2H$ (L)	O	H	OH
SAmM	$-CH_2CH_2CH(NH_2)CO_2H$ (L)	CH_2	OH	OH

Chart II. Analogues of SAM with Modification in the Base Portion



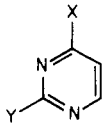
(L form)

purine derivatives, R =



Compd	X	Y	Z	Q	K
SIM	N	OH	H	CH	N
SGM	N	OH	NH_2	CH	N
STM	N	NH_2	H	CH	CH
8-aza-SAM	N	NH_2	H	N	N
N^6-Me-SAM	N	$NHCH_3$	H	CH	N
N^6-Me_2-SAM	N	$N(CH_3)_2$	H	CH	N
3-deaza-SAM	CH	NH_2	H	CH	N
N^6-Me-3-deaza-SAM	CH	$NHCH_3$	H	CH	N
N^6-Me_2-3-deaza-SAM	CH	$N(CH_3)_2$	H	CH	N

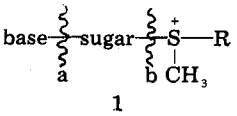
pyrimidine derivatives, R =



Compd	X	Y
SUM	OH	OH
SCM	NH_2	OH

properties (uv and NMR), and by degradation experiments.

The degradation procedure used was that previously described by Zappia et al.[8] for sulfonium nucleosides. Hydrolysis at the sugar–base linkage of the sulfonium nucleoside 1 (split a) was effected by 0.1 N NaOH at 25° for 10 min; with the same hy-



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drolytic conditions at 100°, split b occurred in addition to split a. Under the mild hydrolysis conditions (0.1 N NaOH, 25°, 10 min), the hydrolyzed base portion of the sulfonium nucleoside could be identified by comparison with standard samples on thin-layer chromatography. Under the more stringent hydrolysis conditions, both the base and the amino acid portions could be identified. All the new SAM analogues were characterized by this degradation technique and the results were consistent with the assigned structures.

Table I. R_f Values for SAM Analogues on Thin-Layer Chromatography

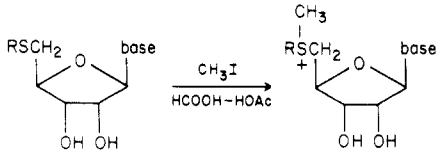
Compd	R_f values and chromatography system[a] A	B	C	D
L-SAM	0.08	0.02	0.10	0.32
D-SAM	0.08	0.02	0.10	0.32
S-Me-SAC	0.16	0.08	0.19	0.32
Decarboxylated SAM	0.13	0.09	0.10	0.42
Deaminated SAM	0.25	0.26	0.24	0.55
N^{α}-Ac-SAM	0.21	0.15	0.14	0.46
2′-Deoxy-SAM	0.11	0.04	0.16	0.35
3′-Deoxy-SAM	0.08	0.02	0.09	0.32
SAmM	0.10	0.03	0.11	0.35
SIM	0.08	0.02	0.10	0.30
SGM	0.08	0.02	0.10	0.30
STM	0.14	0.06	0.08	0.40
8-Aza-SAM	0.16	0.12	0.17	0.35
N^6-Me-SAM	0.09	0.09	0.08	0.50
N^6-Me_2-SAM	0.06	0.09	0.08	0.60
3-Deaza-SAM	0.05	0.02	0.08	0.34
N^6-Me-3-deaza-SAM	0.05	0.06	0.10	0.40
N^6-Me_2-3-deaza-SAM	0.05	0.06	0.09	0.50
SUM	0.19	0.08	0.10	0.30
SCM	0.11	0.03	0.08	0.30

[a] The chromatography systems had the following compositions: system A, 9 parts of EtOH–HOAc–H_2O (50:3:5) and 1 part of pH 7.0, 0.2 M PO_4^{2-} buffer on silica gel GF (250 μ, Analtech); system B, solvent same as system A but run on Avicel F (250 μ, Analtech); system C, *n*-BuOH–H_2O–HOAc (10:9:3) on silica gel GF (250 μ, Analtech); system D, solvent same as system C but run on Avicel F (250 μ, Analtech).

Further evidence consistent with the assigned structures for the SAM analogues was the NMR spectra using the corresponding SAH analogues as references. A characteristic signal (δ 2.80–3.20) for the methyl group at the sulfonium center was observed for each of the SAM analogues prepared.

General Procedure for the Conversion of SAH Analogues to the Corresponding Isotopically Labeled SAM-$^{14}CH_3$ Analogues. Preparation of the ^{14}C-labeled SAM analogues was accomplished by a modification of the procedure outlined above. Commercially available $^{14}CH_3I$ (500 μCi, specific activity = 55 mCi/mmol) was diluted to a specific activity of 0.5 mCi/mmol by adding unlabeled CH_3I, which was dissolved in HCOOH (0.225 ml) and glacial HOAc (0.225 ml). To 0.10 ml (100 μCi, $^{14}CH_3I$,

Scheme I



base = adenine, guanine, hypoxanthine, uracil, cytosine, 3-deazaadenine, 7-deazaadenine, etc.; R = $-CH_2CH_2CH(NH_2)CO_2H$ (D or L), $-CH_2CH_2CH_2NH_2$, etc.

specific activity 0.5 mCi/mmol) of this stock solution was added 5 mg of a structural analogue of SAH. The reaction mixture was allowed to stand in the darkness for 5 days after which time the product was isolated and purified as described above for the unlabeled SAM analogues. For the step involving ion-exchange chromatography, very small columns (0.5 × 5 cm) containing Amberlite IRC-50 ion-exchange resin were utilized. The specific activities of the isolated $^{14}CH_3$-labeled SAM analogues were approximately 0.4 mCi/mmol (1000 dpm/nmol). The $^{14}CH_3$-labeled SAM analogues were characterized by their thin-layer chromatographic properties using the unlabeled SAM analogues as standards and by their uv spectra from which the molar concentrations were determined.

Enzyme Preparation and Assay. The enzymes used in this study were purified from the following sources according to previously described procedures: COMT,[10,19] rat liver (male, Sprague–Dawley, 180–200 g), PNMT,[10,20] bovine adrenal medulla (Pel-Freez Biologicals); HMT,[10,21] guinea pig brain (Pel-Freez Biologicals); and HIOMT,[10,22] bovine pineal glands (Pel-Freez Biologicals). COMT, PNMT, HMT, and HIOMT were assayed using radiochemical techniques as described in the preceding papers of this series.[10–15]

The unlabeled SAM analogues prepared in this study were tested as inhibitors of the transmethylations from L-SAM-$^{14}CH_3$ to the appropriate acceptor molecules which are catalyzed by COMT, PNMT, HMT, and HIOMT. The procedures used in this study were identical with those described earlier in out studies of the inhibition of methyltransferases by SAH analogues.[10–15] Processing of the kinetic data was achieved as previously described.[10–15,23,24]

The isotopically labeled SAM-$^{14}CH_3$ analogues were evaluated for their potential to donate a methyl group in the transmethylations catalyzed by COMT, PNMT, HMT, and HIOMT. The enzymatic assays were carried out in a manner identical with that described previously,[10–15] except that the appropriate SAM-$^{14}CH_3$ analogues were used as methyl donors in the incubation mixtures instead of SAM-$^{14}CH_3$.

Results and Discussion

Chemistry. The desired SAM analogues listed in Charts I and II were prepared either unlabeled or isotopically labeled by methylation of the corresponding SAH analogues[10–15] using CH_3I or $^{14}CH_3I$ in a mixture of formic acid and acetic acid as shown in Scheme I.

As in the case of the chemical synthesis of SAM previously described by Jamieson,[16] the SAM analogues prepared in this study were racemic at the sulfonium center. Therefore, the sulfonium derivatives of SAM obtained in these studies should be considered as (±) diastereoisomers. These SAM analogues (Charts I and II) were characterized by their NMR and uv spectral properties, their chromatographic properties, and by the products resulting from hydrolysis under basic conditions. The chemical instability of these SAM analogues prohibited the usual crystallization and characterization by chemical analyses. The R_f values for the various SAM analogues using four thin-layer chromatography systems are listed in Table I. Before samples were used for enzyme studies, they were carefully checked to assure no contamination by the corresponding SAH analogue.

The degradation studies of the new sulfonium compounds were carried out using the procedure previously described by Zappia et al.[8] (see Experimental Section). This technique takes advantage of the sensitivity of SAM and its analogues to base hydrolysis and the characteristic hydrolytic pattern one observes with such nucleoside sulfonium compounds. For example, base hydrolysis (0.1 N NaOH, 25°, 10 min) of 3-deaza-SAM resulted in the split of the sugar–base glycosidic linkage into 3-deazaadenine and S-ribosylmethionine. Under the same hydrolytic conditions at 100°, hydrolysis occurred not only at the sugar–base glycoside linkage to give 3-deazaadenine but also at the sulfonium center to yield methionine. These hydrolysis products could be easily identified by thin-layer chromatography. These degradation techniques provide a simple method to (1) test for a sulfonium nucleoside, since the corresponding SAH analogues are not sensitive to hydrolysis under these conditions, and (2) provide a means of structural proof, since the hydrolysis products are easily identifiable. Such hydrolytic data were obtained for each of the SAM analogues prepared in this study.

SAM Analogues as Inhibitors of Methyltransferases in Vitro. The various unlabeled analogues of SAM which were synthesized as part of this study (Charts I and II) were tested as inhibitors of COMT, PNMT, HMT, and HIOMT. The preliminary results are shown in Table II.

Table II. Inhibition of COMT, PNMT, HMT, and HIOMT by the Unlabeled Analogues of SAM[a]

Compd[b]	Inhibitor[c] concn, mM	% inhibition COMT	PNMT	HMT	HIOMT
D-SAM	0.2	1	0	36	0
	2.0	9	19	94	12
S-Me-SAC	0.2	2	2	10	28
	2.0	8	19	36	36
Decarboxylated SAM	0.2	4	4	14	0
	2.0	6	15	33	0
Deaminated SAM	0.2	0	0	11	5
	2.0	0	2	16	12
N^a-Ac-SAM	0.2	0	0	5	0
	2.0	9	16	9	0
2'-Deoxy-SAM	0.2	5	4	9	0
	2.0	16	19	28	0
3'Deoxy-SAM	0.2	8	11	10	3
	2.0	38	51	69	35
SIM	0.2	3	2	1	0
	2.0	10	12	12	9
SGM	0.2	2	4	9	8
	2.0	6	9	19	30
STM	0.2	37	45	23	8
	2.0	88	91	92	54
8-Aza-SAM	0.2	2	6	9	8
	2.0	24	17	30	54
N^6-Me-SAM	0.2	1	0	7	0
	2.0	26	16	10	5
N^6-Me_2-SAM	0.2	0	0	4	4
	2.0	25	0	9	8
3-Deaza-SAM	0.2	3	21	17	6
	2.0	20	77	74	41
N^6-Me-3-deaza-SAM	0.2	3	1	4	5
	2.0	20	10	24	16
N^6-Me_2-3-deaza-SAM	0.2	0	0	0	0
	2.0	2	0	0	0
SUM	0.2	4	0	1	8
	2.0	12	10	18	18
SCM	0.2	8	0	2	0
	2.0	39	3	13	0

[a] COMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section, except in each case the SAM concentration = 1.0 mM. [b] All SAM analogues were racemic at the sulfonium center; therefore, they were a mixture of (±) diastereoisomers. [c] The inhibitors were prepared in aqueous stock solutions (10.0 μmol/ml).

Table III. Inhibition Constants for Analogues of SAM toward COMT, PNMT, HMT, and HIOMT[a]

Inhibitors	Inhibn constants, μM,[b,c] K_{is} ± SEM			
	COMT	PNMT	HMT	HIOMT
D-SAM	810 ± 169	779 ± 18.3	92.2 ± 15.2	
Decarboxylated SAM			1094 ± 450.5	
3′-Deoxy-SAM		599 ± 48	458 ± 100	
STM	17 ± 1.7	26.3 ± 2.48	28 ± 4	110 ± 7.1
8-Aza-SAM	982 ± 238	1671 ± 300		392 ± 45.9
N^6-Me-SAM	310 ± 43.7		1218 ± 443	
3-Deaza-SAM	491 ± 62.7	58 ± 3.0	93 ± 13	205 ± 14.0
N^6-Me-3-deaza-SAM				1419 ± 389

[a] COMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section except in each the SAM concentration was 24–210 μM. [b] Each inhibitor showed linear competitive kinetics.[24] [c] When low inhibitory activity was observed from the preliminary studies (Table II), no extensive studies were done to determine the kinetic inhibitor constants.

Using reciprocal velocity vs. reciprocal L-SAM concentration plots, the kinetic patterns for inhibition of COMT, PNMT, HMT, and HIOMT by the active analogues (D-SAM, decarboxylated SAM, 3′-deoxy-SAM, STM, N^6-Me-SAM, 3-deaza-SAM, N^6-Me-3-deaza-SAM, and 8-aza-SAM) were determined and resulting inhibition constants are given in Table III. In all cases linear competitive patterns of inhibition were observed when L-SAM was the variable substrate.

The methyltransferases tested, in general, showed very strict specificity for the amino acid portion of SAM. The interesting exception was HMT for which D-SAM was found to be a potent inhibitor (K_{is} = 92.2 ± 15.2 μM). In contrast, D-SAM was nearly inactive toward COMT, PNMT, and HIOMT. These observations are consistent with the results obtained earlier with D-SAH, which was also found to be inactive as an inhibitor with most methyltransferases except for HMT.[10] The other amino acid modified analogues of SAM (S-Me-SAC, decarboxylated SAM, deaminated SAM, and N^{α}-Ac-SAM) were essentially inactive as inhibitors of these enzymes. These observations indicate that the terminal amino and carboxyl groups present in the L configuration and the three-carbon distance between the terminal groups and the sulfonium center are absolute requirements for the proper binding of the SAM molecule to COMT, PNMT, and HIOMT. For HMT, all of the structural features on the amino acid portion of SAM listed above except the chirality of α-carbon are crucial for binding. These results are consistent with the binding requirements observed previously for the amino acid modified SAH analogues.[10]

The enzymes showing the strictest specificity for the 2′- and 3′-hydroxy groups of the sugar portion of SAH were COMT and HIOMT, since 2′-deoxy-SAM and 3′-deoxy-SAM showed only weak inhibitory activity toward these enzymes. The results for the sugar-modified SAM analogues with HIOMT are consistent with those results observed with the corresponding sugar-modified SAH analogues.[12] However, with COMT, the poor inhibitory activity of 3′-deoxy-SAM is different from that observed for the corresponding 3′-deoxy-SAH, which was shown in our earlier studies to be a good inhibitor of COMT (K_i = 138 ± 31 μM).[12] Similarly, 2′-deoxy-SAM and 3′-deoxy-SAM were observed to be substantially less active as inhibitors of PNMT than their corresponding demethylated products 2′-deoxy-SAH and 3′-deoxy-SAH. These results indicate that 2′- and 3′-hydroxy groups of SAM may play a much more important role in the binding of SAM to PNMT than in the binding of SAH.[12] It is extremely interesting to note that 3′-deoxy-SAM (K_i = 458 ± 100 μM) is a weak inhibitor of HMT but significantly better as an inhibitor than the corresponding 3′-deoxy-SAH (K_i = 2070 ± 864 μM).[12] This observation would suggest that at the binding site of HMT the 3′-hydroxyl group plays a less significant role in the binding of L-SAM than in the binding of L-SAH. These results appear to be a reversal of the trend observed above for COMT and PNMT.

The role of the 2′- and 3′-hydroxy groups of SAM in enzymatic binding is at this point unclear. They may be involved either in maintaining the proper orientation of the amino acid and base portions or in direct binding with amino acid residues (e.g., hydrogen binding). There is good evidence in the literature to suggest that there are differences in the conformation of the ribose and 2′-deoxyribose ring systems. It has been reported that the ribose ring system in adenosine is puckered with the carbon bearing the 3′-hydroxyl group projecting out of the plane (endo) formed by the other four atoms of the sugar ring and lying on the same side of the plane as the 5′-carbon and the adenine base. In contrast to adenosine, 2′-deoxyadenosine is puckered with the 3′-hydroxyl group lying on the opposite side of the plane as the 5′-carbon and the adenine base (exo). When the out-of-plane atom in the ribofuranosides is endo (C-3′ in the case of adenosine), the hydroxyl oxygen attached to this carbon falls almost directly into the four-atom ring plane (equatorial). But if the puckering is such that the displaced atom is exo, as in 2′-deoxyadenosine, then the oxygen is directed from the plane of the ring (axial). Furthermore, the relative distances between C-5′ and C–N glycosidic linkage are different in adenosine and 2′-deoxyadenosine.

It is possible that the positive charge on the sulfonium center of L-SAM may have an effect on the puckering of the ribose, 2′-deoxyribose, or 3′-deoxyribose ring systems. Thus the relative orientation of the amino acid and base portions in SAM as compared to SAH may be effected differently. Because 2′-deoxy-SAM and 3′-deoxy-SAM are substantially weaker inhibitors of COMT and PNMT than the corresponding SAH analogues, the indication is that the introduction of the positive charge at the sulfur atom has adversely affected some of the interactions critical to the formation of the E–I complex. One possible explanation is a difference in the conformation of the ribose ring system.

In general, the inhibitory activities of the base-modified analogues of L-SAM (Tables II and III) were consistent with those for the corresponding SAH analogues. The weak inhibitory activities of SUM and SCM indicate the importance of the purine ring system of L-SAM in enzymatic binding, whereas the importance of the 6-amino group of the adenine portion of SAM is indicated by the poor inhibitory activity of SIM, SGM, N^6-Me-SAM, N^6-Me_2-SAM, N^6-Me-3-deaza-SAM, and N^6-Me_2-3-deaza-SAM toward all of the enzymes tested. These results are consistent with our earlier observations with SAH analogues.[11] Also consistent with our earlier observations[11]

Table IV. Michaelis-Menten Constants for Analogues of SAM as Substrates of COMT, PNMT, HMT, and HIOMT[a]

Compd	Kinetic constants[b]	COMT	PNMT	HMT	HIOMT
(−)-L-SAM	K_m, μM ± SEM	9.66 ± 0.744	8.66 ± 2.11	2.54 ± 0.26	6.86 ± 2.61
	V_{max} ± SEM[c]	17.85 ± 0.46	4.50 ± 0.35	1.13 ± 0.02	0.115 ± 0.014
D-SAM	K_m, μM ± SEM			93 ± 7.85	
	V_{max} ± SEM[c]			0.38 ± 0.02	
3′-Deoxy-SAM	K_m, μM ± SEM	337 ± 34	134 ± 24	220 ± 24	
	V_{max} ± SEM[c]	48.1 ± 3.12	1.51 ± 0.17	0.71 ± 0.04	
SAmM	K_m, μM ± SEM	125 ± 62	24 ± 1.2	81 ± 19.3	53 ± 3.1
	V_{max} ± SEM[c]	1.49 ± 0.38	2.35 ± 0.31	0.41 ± 0.04	0.197 ± 0.004
STM	K_m, μM ± SEM	135 ± 15	65 ± 3.6	43.2 ± 5.3	73 ± 2.75
	V_{max} ± SEM[c]	24 ± 1.3	0.90 ± 0.18	0.23 ± 0.01	0.50 ± 0.01
8-Aza-SAM	K_m, μM ± SEM	1170 ± 278	473.5 ± 114	143.5 ± 15	124.5 ± 10.9
	V_{max} ± SEM[c]	166 ± 31.8	4.94 ± 0.77	1.03 ± 0.05	0.78 ± 0.04
N^6-Me-SAM	K_m, μM ± SEM	348.5 ± 52	186.5 ± 14	179.5 ± 25	
	V_{max} ± SEM[c]	40 ± 4.0	2.61 ± 0.11	0.91 ± 0.01	
3-Deaza-SAM	K_m, μM ± SEM	635 ± 50	20.8 ± 1.75	25.8 ± 12.7	48.5 ± 1
	V_{max} ± SEM[c]	85 ± 5.1	0.30 ± 0.05	0.11 ± 0.02	0.22 ± 0.02
N^6-Me-deaza-SAM	K_m, μM ± SEM		212 ± 20.3	633 ± 137	
	V_{max} ± SEM[c]		2.98 ± 0.15	2.68 ± 0.43	

[a] COMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section. [b] Reciprocal velocities were plotted graphically against reciprocals of the substrate concentrations as shown in Figure 1. Since the synthetic SAM analogues were mixtures of the (±) sulfonium isomers and since we had shown that only the (−) isomer was active as a methyl donor, the substrate concentrations plotted were half of that calculated from the uv spectra. Therefore, the K_m values calculated should be treated as apparent K_m values, since the (+) isomer may produce inhibition and alter the binding of the (−) isomer. The data in the linear region of these plots were used to calculate the K_m and V_{max} using a least-squares method. [c] nmol of product formed/mg of protein/min.

was the finding that minor modifications of the adenine ring system of L-SAM can be tolerated by methyltransferases, as long as the modified purine base contains a 6-amino group. For example, STM and 3-deaza-SAM were fairly potent inhibitors of all of the enzymes studied (Table III).

Of particular interest was 8-aza-SAM, since a significant difference in the inhibitory behavior was observed for this analogue when compared to the corresponding 8-aza-SAH analogue. In earlier studies,[14] we had shown 8-aza-SAH to be a fairly specific, potent inhibitor of HIOMT (K_i = 18.5 ± 3.68 μM). However, as shown in Table III, conversion of 8-aza-SAH to the corresponding 8-aza-SAM analogues results in a substantial decrease in its inhibitory activity toward HIOMT (K_i = 392 ± 45.9 μM).

SAM Analogues as Substrates for Methyltransferases in Vitro. The various $^{14}CH_3$-labeled SAM analogues which were synthesized as part of this study were evaluated as substrates for COMT, PNMT, HMT, and HIOMT. Those $^{14}CH_3$-labeled SAM analogues which showed methyl-donating capabilities in preliminary experiments were further evaluated by determining the Michaelis–Menten constants (K_m and V_{max}). If the K_m and V_{max} values are not reported in Table IV for a specific SAM analogue, this indicates that the analogue showed no methyl-donating capabilities in the enzyme-catalyzed reactions studied. Only data for the SAM analogues showing methyl-donating activity are reported in Table IV. Shown in Figure 1, as examples, are reciprocal velocity vs. reciprocal substrate concentration plots for SAmM, STM, 3-deaza-SAM, 8-aza-SAM, and L-SAM itself with PNMT. In all cases reasonably linear plots were obtained for each active analogue tested. Similar results for these SAM analogues were also obtained with COMT, HMT, and HIOMT. From these reciprocal plots, Michaelis–Menten constants (K_m and V_{max}) were calculated using a least-squares method (see Experimental Section).

Similar to the results obtained earlier with (±)-L-SAM,[7] we have also observed that two of the SAM analogues (STM and 3-deaza-SAM) donate their methyl groups from only one of the two sulfonium isomers. In experiments similar to those described earlier with (±)-L-SAM,[7] we found that approximately 50% of the isotopically labeled

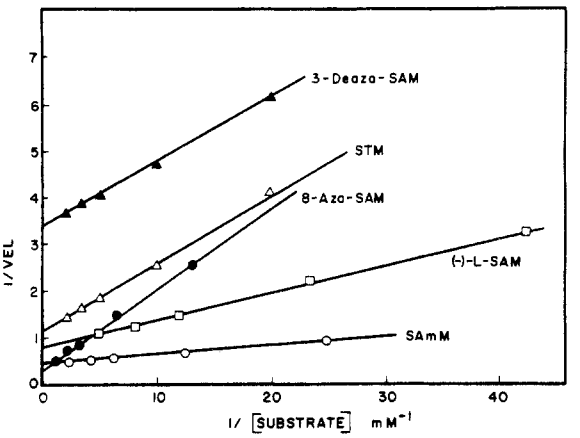


Figure 1. Reciprocal velocity vs. reciprocal substrate plots for active analogues of SAM toward PNMT. The enzyme assay conditions are described in the Experimental Section. Vel = nmol of product/mg of protein/min. See Table IV for the calculated K_m and V_{max} values.

methyl groups are transferred from (±)-STM and (±)-3-deaza-SAM in transmethylation reactions catalyzed by COMT, PNMT, HMT, and HIOMT. Therefore, in the experiments carried out to determine K_m and V_{max} values for the SAM analogues (Table IV), we have assumed that only the (−) isomer is capable of donating its methyl group in these reactions. The K_m values reported in Table IV should, therefore, be treated as apparent K_m values, since the presence of the corresponding (+) isomers may produce inhibitory effects on these transmethylation reactions and, therefore, result in artificially high K_m values.

All of the amino acid modified analogues of SAM except D-SAM were found to be inactive as substrates for the methyltransferases tested. With HMT, D-SAM showed methyl donor activity with a slightly lower V_{max} and a K_m value substantially higher than that for the L isomer (Table IV). D-SAM was not a substrate for COMT, PNMT, or HIOMT.

With the sugar-modified analogues, SAmM was found to be as good a substrate as L-SAM for PNMT. SAmM showed methyl donor activity for PNMT with a V_{max} approximately equal to that of L-SAM, but with a slightly

higher K_m value. In addition, SAmM was a fairly good substrate for HMT, COMT, and HIOMT, but with substantially higher K_m values than the natural methyl donor. SAmM is an analogue of SAM in which the 1′,5′-oxygen linkage is replaced by a methylene group. This modification of the sugar moiety may have an effect on the orientation of the sulfonium center of SAmM as compared to the sulfonium center in SAM.

Among the other sugar-modified SAM analogues studied, 2′-deoxy-SAM was shown to be inactive as a substrate for the methyltransferases tested. 3′-Deoxy-SAM showed methyl donor activities with maximal rates (V_{max}'s) closely paralleling those of L-SAM in the COMT-, PNMT-, and HMT-catalyzed reactions. However, these methyltransferases showed less affinity for 3′-deoxy-SAM than for L-SAM, which was consistent with the inhibitory activities of this compound. 3′-Deoxy-SAM was not a substrate for the HIOMT-catalyzed reaction.

In the base-modified analogues, replacement of the adenine moiety of L-SAM with pyrimidine bases (e.g., SUM, SCM) or other purine bases, which lack a 6-amino group (e.g., SGM, SIM), resulted in complete loss of methyl donor activities. Further support for the requirement of the 6-amino group of adenine in binding of SAM can be seen by the sharp decrease in affinities (increases in K_m's) of the methyltransferases for N^6-Me-SAM and N^6-Me-3-deaza-SAM relative to L-SAM and 3-deaza-SAM. Interestingly, both N^6-Me-SAM and N^6-Me-3-deaza-SAM donated their methyl groups at similar or higher rates (V_{max}'s) than SAM for the reactions catalyzed by PNMT and HMT. Both of these N^6-methylated SAM analogues were inactive as methyl donors in the HIOMT-catalyzed reaction. Furthermore, the corresponding tertiary amino derivatives of SAM (N^6-Me_2-SAM and N^6-Me_2-3-deaza-SAM) were completely inactive as substrates for the methyltransferases tested.

Some of the more interesting SAM analogues were STM, 8-aza-SAM, and 3-deaza-SAM. For example, STM was a fairly good substrate for all of the methyltransferases tested. Replacement of the 7-nitrogen in the adenine portion of SAM by carbon appears to have only a slight effect on methyl transfer from this substrate. Only slight differences in the V_{max} values of STM and SAM in these transmethylation reactions were noted. However, substantial differences in the K_m values were observed. In general, 3-deaza-SAM was also a good substrate for the methyltransferases tested, although it had a substantially higher K_m for COMT. This finding confirms our observations noted earlier that COMT had a lower affinity for 3-deaza-SAM than for 3-deaza-SAH. 8-Aza-SAM was shown to donate its methyl group at the same rate or substantially higher rates (V_{max}'s) than L-SAM with the various methyltransferases tested. However, this analogue had in general poor affinity (high K_m's) for COMT, PNMT, HMT, and HIOMT. These results were in agreement with the data obtained for the unlabeled 8-aza-SAM as an inhibitor toward these enzymes.

Conclusions

In this study we have attempted to elucidate the structural features of L-SAM which are required for the binding of this molecule to the active sites of COMT, PNMT, HMT, and HIOMT. The chemical syntheses of various labeled ($^{14}CH_3$) and unlabeled analogues of L-SAM have been achieved in this study. Using the unlabeled analogues of L-SAM, inhibition studies have been carried out and the inhibition constants (K_{is}) for the active SAM analogues have been determined (Tables II and III). The methyl donor activities of various labeled ($^{14}CH_3$) analogues of L-SAM have also been determined and the Michaelis–Menten constants (K_m and V_{max}) are reported (Table IV). Of particular interest was the observation that in addition to the natural methyl donor, some of these synthetic SAM analogues showed comparable methyl donor activities in the transmethylation reactions studied.

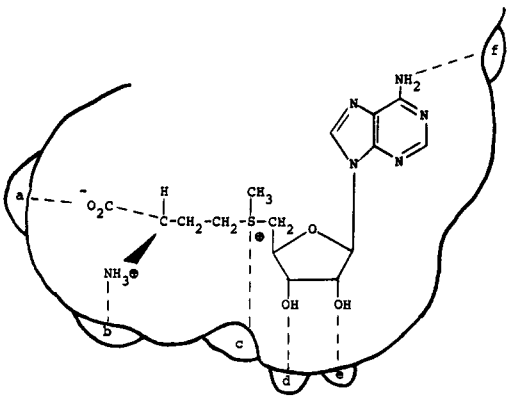


Figure 2. Proposed enzymatic binding sites for SAM.

In general, the structure–activity relationships for the inhibitory activities and methyl donor activities of the SAM analogs toward these methyltransferases were in agreement with the requirements for binding of the corresponding L-SAH analogues.[10–15] There are, however, some interesting exceptions, particularly with respect to the role of the 2′- and 3′-hydroxy groups in enzymatic binding of SAH and SAM. From the results of this study, it can be concluded that the role of the 2′- and 3′-hydroxy groups of SAM in enzymatic binding is substantially more important than the role these groups play in the binding of SAH. Alterations in the 2′- or 3′-hydroxy groups of SAM greatly affected the binding to COMT, HIOMT, PNMT, and HMT. This is demonstrated by the weak inhibitory activities (K_{is}) of 2′-deoxy-SAM and 3′-deoxy-SAM toward these transmethylation reactions. It is interesting to note, however, that 3′-deoxy-SAM showed a potential to donate its methyl group in the COMT, PNMT, and HMT catalyzed reaction at a maximum rate (V_{max}) comparable to L-SAM. This would tend to suggest that when 3′-deoxy-SAM binds to these enzymes the requirements for the methyl-transfer reactions are satisfied, but the affinity of these enzymes for 3′-deoxy-SAM is substantially lower than for SAM, suggesting loss of some factors crucial for maximum enzyme–ligand binding.

In general, it can be concluded that methyltransferases show very strict specificity for the structural features of L-SAM. In fact, it would appear that the specificity for L-SAM is much stricter in several cases than the specificity for L-SAH. For example, in earlier studies[12] we had shown that sugar-modified analogues of SAH (2′-deoxy-SAH and 3′-deoxy-SAH) were potent inhibitors of PNMT; however, in this study we found that 2′-deoxy-SAM was inactive as an inhibitor and 3′-deoxy-SAM showed only weak inhibitory activity toward this enzyme. Similarly, we had previously observed that 8-aza-SAH was a potent inhibitor of HIOMT;[14] however, the corresponding 8-aza-SAM was only a weak inhibitor of this enzyme. A similar trend was observed with 3-deaza-SAH vs. 3-deaza-SAM as inhibitors of COMT. An exception to this trend appears to be STM which showed inhibitor activity toward COMT, PNMT, HMT, and HIOMT comparable to STH.[14]

Figure 2 shows a schematic representation of a possible enzymatic binding site for SAM. For interaction of SAM with COMT, PNMT, and HIOMT, the terminal carboxyl (site a), the terminal amino (site b), the configuration of

the amino acid asymmetric carbon, and the three-carbon distance between the sulfonium center and the asymmetric amino acid carbon are all absolute requirements for maximum enzymatic binding and for maximum potential as a methyl donor. The configuration of the sulfonium center of SAM (site c) appears to play an absolutely critical role in determining whether a compound will serve as a methyl donor; however, the configuration at this center does not appear to affect enzymatic binding.[7] In the binding of L-SAM, the 3′-hydroxyl (site d) and the 2′-hydroxyl (site e) groups of the ribose moiety are extremely crucial. In contrast, the 1′,5′-oxygen bridge of the ribose portion of SAM appears not to be crucial for binding or methyl-donating capabilities with these enzymes. In the base portion of L-SAM, the 6-amino group (site f) is an absolute requirement for the binding and methyl-donating properties of SAM. With COMT, PNMT, and HIOMT the only modification in the structure of SAM which the enzymes will tolerate are minor changes in the base portion. For example, 3-deaza-SAM and STM are potent inhibitors of these methyltransferases.

For the interaction of SAM with HMT all of the functional groups shown in Figure 2, except the configuration of the amino acid asymmetric carbon, are required to produce maximum binding and maximum potential as a methyl donor. This result is somewhat consistent with earlier data for the inhibitory effects of D-SAH.[11] However, it appears that the configuration at this asymmetric center is more crucial in the binding of SAM than in the binding of SAH, since D-SAH (K_{is} = 10.5 ± 1.3 μM) was a much more potent inhibitor of HMT than D-SAM (K_{is} = 92.2 ± 15.2 μM).

Therefore, it would appear from the results of these studies that the structural requirements for binding SAM are similar but much more stringent than for binding SAH. In addition, from the standpoint of designing inhibitors based on the SAM molecule vs. the SAH molecule, pursuing modifications of the basic SAH structure appears to have many advantages. The most obvious advantage is that the structural requirements for binding SAH are not as strict as for binding SAM which permits more flexibility for possible structural changes. But even more importantly, the difference in the specificity of the SAH binding sites, which have been detected in our earlier studies,[12-17] does not appear to hold true for the binding of SAM. Without these differences in specificity between methyltransferases, the development of SAH and SAM analogues capable of selectively inhibiting a single enzyme without affecting all SAM-dependent methyltransferases would be impossible.

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References and Notes

(1) Established Investigator of the American Heart Association.
(2) Abbreviations used are L-SAM, *S*-adenosyl-L-methionine; L-SAH, *S*-adenosyl-L-homocysteine; D-SAM, *S*-adenosyl-D-methionine; *S*-Me-SAC, *S*-adenosyl-L-(2-amino-3-methylthio)propionic acid; decarboxylated SAM, *S*-adenosyl-3-methylthiopropylamine; deaminated SAM, *S*-adenosyl-4-methylthiobutyric acid; N^{α}-Ac-SAM, *S*-adenosyl-L-*N*-acetylmethionine; 2′-deoxy-SAM, *S*-2′-deoxyadenosyl-L-methionine; 3′-deoxy-SAM, *S*-3′-deoxyadenosyl-L-methionine; SAmM, *S*-aristeromycinyl-L-methionine; SIM, *S*-inosyl-L-methionine; SGM, *S*-guanosyl-L-methionine; STM, *S*-tubercidinyl-L-methionine; 8-aza-SAM, *S*-8-azaadenosyl-L-methionine; N^6-Me-SAM, *S*-N^6-methyladenosyl-L-methionine; N^6-Me_2-SAM, *S*-N^6-dimethyladenosyl-L-methionine; 3-deaza-SAM, *S*-3-deazaadenosyl-L-methionine; N^6-Me-3-deaza-SAM, *S*-N^6-methyl-3-deazaadenosyl-L-methionine; N^6-Me-3-deaza-SAM, *S*-N^6-dimethyl-3-deazaadenosyl-L-methionine; SUM, *S*-uridyl-L-methionine; SCM, *S*-cytidyl-L-methionine; COMT, catechol *O*-methyltransferase (E.C. 2.1.1.6); PNMT, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1.28); HMT, histamine *N*-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole *O*-methyltransferase (E.C. 2.1.1.4); K_{is}, inhibition constant for the slope.
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