Potential Inhibitors of S-Adenosylmethionine-Dependent Methyltransferases. 4. Further Modifications of the Amino Acid and Base Portions of S-Adenosyl-L-homocysteine

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Structural analogues of S-adenosyl-L-homocysteine (L-SAH), with modifications in the amino acid or base portions of the molecule, have been synthesized and evaluated for their abilities to inhibit the transmethylations catalyzed by catechol O-methyltransferase (COMT), phenylethanolamine N-methyltransferase (PNMT), histamine N-methyltransferase (HMT), hydroxyindole O-methyltransferase (HIOMT), and indoleethylamine N-methyltransferase (INMT). From these studies some interesting and potentially useful differences in the structural features of L-SAH needed to produce maximal binding to these methyltransferases were detected. This paper provides evidence that 8-azaadenosyl-L-homocysteine is a potent and selective inhibitor of HIOMT, whereas N^6-methyladenosyl-L-homocysteine and N^6-methyl-3-deazaadenosyl-L-homocysteine are selective inhibitors of INMT. In contrast, it was found that S-tubercidinyl-L-homocysteine was a fairly potent, but nonselective inhibitor of all of the methyltransferases studied. The differences and the similarities in the requirements for the binding of SAH to methyltransferases, which were detected in this study and earlier studies from our laboratory, are described. The possibilities of utilizing differences in binding requirements for the design of SAH analogues as specific inhibitors of methyltransferases are discussed.

S-Adenosyl-L-homocysteine (L-SAH)[2] has been shown to be a potent inhibitor of S-adenosylmethionine (SAM)-dependent methyltransferases.[3] The inhibition of SAM-dependent methyltransferases by L-SAH appears to be a general characteristic of this class of enzymes, so that the possibilities of using L-SAH to inhibit specific transmethylation reactions in vivo are limited. In an effort to develop inhibitors specific for a particular enzyme, considerable research interest has been directed in recent years toward the synthesis of SAH analogues.[3] In the preceeding papers in this series[4-6], we have described the synthesis and biological activity of SAH analogues which have structural modifications in the amino acid, sugar, or base portions of the SAH molecule. From the results of these studies, interesting differences in the specificity of the SAH binding sites have been detected, suggesting that differential inhibition of SAM-dependent methyltransferases in vivo using appropriately designed SAH analogues may be possible. For example, various sugar-modified analogues of SAH (e.g., 3'-deoxyadenosyl-L-homocysteine and S-aristeromycinyl-L-homocysteine) are fairly selective inhibitors of phenylethanolamine N-methyltransferase (PNMT),[4c,6] whereas an amino acid modified SAH analogue (S-adenosyl-D-homocysteine) inhibits only histamine N-methyltransferase (HMT).[4a]

In an effort to further characterize the SAH binding sites on catechol O-methyltransferase (COMT), hydroxyindole O-methyltransferase (HIOMT), indoleethylamine N-methyltransferase (INMT), PNMT, and HMT, and thus detect other differences in the specificity of the SAH binding sites, we have carried out further structural modifications of the amino acid and base portions of the SAH molecule (Chart I). The results of these investigations are reported in this paper.

Experimental Section

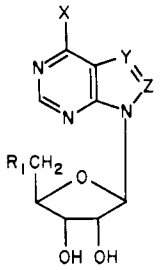
Melting points were obtained on a calibrated Thomas-Hoover Uni-melt and were corrected. Microanalyses were conducted on an F & M Model 185 C, H, N analyzer, the University of Kansas, Lawrence, Kan. Unless otherwise stated, the ir, NMR, and uv data were consistent with the assigned structures. Ir data were recorded on a Beckman IR-33 spectrophotometer, NMR data on a Varian Associates Model T-60 spectrophotometer (Me_4Si), 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). Spots were detected by visual examination under uv light and/or ninhydrin for compounds containing amino moieties.

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/ml and stored at −20 °F. SAM chloride (Sigma) was stored as a 10-mM aqueous stock solution. The following compounds were commercially available from the indicated sources: tetra-O-acetyl-D-ribofuranose (Pfanstiehl); 3,4-dihydroxybenzoate, 8-azaadenine (Aldrich); DL-β-phenylethanolamine, histamine dihydrochloride, N-acetylserotonin, S-benzyl-L-cysteine, D-homocystine, L-homocystine, L-cystine, tubercidin, N^6-methyladenosine, and N^6-dimethyladenosine (Sigma). L-SAH and D-SAH were prepared by reaction of 5'-chloro-5'-deoxyadenosine with L-homocystine and D-homocystine, respectively, as previously described.[7] N^α-Ac-D-SAH was prepared from D-SAH and Ac_2O using a procedure identical with that described earlier by Hildesheim et al.[8] for the preparation of N^α-Ac-L-SAH. D-SAH sulfoxide was prepared by oxidation of D-SAH with 30% hydrogen peroxide as described earlier for the preparation of L-SAH sulfoxide.[4a]

S-Adenosyl-L-cysteine (L-SAC). (A) From 5'-Chloro-5'-deoxyadenosine and L-Cystine. 5'-Chloro-5'-deoxyadenosine was prepared in 75% yield from adenosine (1.0 g, 3.74 mmol) and thionyl chloride (2.0 ml) in hexamethylphosphoramide (10 ml) by a modification[7] of the procedure of Kikugawa and Ichino.[9] To L-cystine (254 mg, 1 mmol) in 15 ml of liquid NH_3 was added Na in small pieces until a blue color was maintained for 20 min. To this solution of L-cysteine anion was added 5'-chloro-5'-deoxyadenosine (286 mg, 1 mmol) and the resulting mixture stirred vigorously at −70° for 5 h. The NH_3 was allowed to slowly evaporate after which the solid residue was dissolved in 10 ml of H_2O and the resulting aqueous solution was neutralized to pH 7.0 with 1.0 N H_2SO_4. The product was purified by ion-exchange chromatography (Dowex 50W-X4, 50–100 mesh, NH_4^+ form) eluting with H_2O followed by 1 N NH_4OH. The fractions containing product were concentrated in vacuo and lyophilized. The lyophilized sample of L-SAC was crystallized (EtOH–H_2O) to yield 189 mg (50%): mp 215–218° (lit.[8] 223–224° dec).

(B) From 2',3'-Isopropylidene-5'-O-toluene-p-sulfonyladenosine and S-Benzyl-L-cysteine. In addition to the procedure outlined above, L-SAC was also prepared by the condensation of 2',3'-O-isopropylidene-5'-O-toluene-p-sulfonyladenosine with S-benzyl-L-cysteine similar to the procedure previously reported by Hildesheim et al.[8] 2',3'-O-Isopropylidene-5'-O-toluene-p-sulfonyladenosine[4a] (1.1 g, 2.38 mmol) was allowed to react with S-benzyl-L-cysteine (0.4 g, 1.89 mmol) in Na and liquid NH_3 to yield the intermediate 2',3'-isopropylidene-L-SAC. The 2',3'-isopropylidene-L-SAC was purified by thick-layer chromatography on cellulose (Analtech, 1000 μ) eluting with EtOH–H_2O (3:2). The product was removed from the cellulose by extraction with H_2O followed by lyophilization to yield 2',3'-isopropylidene-L-SAC which was homogeneous on TLC [silica

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



Compd	R_1	X	Y	Z
L-SAH	$-SCH_2CH_2CH(NH_2)CO_2H$ (L)	NH_2	N	CH
D-SAH sulfoxide	$-S(\rightarrow O)CH_2CH_2CH(NH_2)CO_2H$ (D)	NH_2	N	CH
N^{α}-Ac-D-SAH	$-SCH_2CH_2CH(NHAc)CO_2H$ (D)	NH_2	N	CH
L-SAC	$-SCH_2CH(NH_2)CO_2H$ (L)	NH_2	N	CH
STH	$-SCH_2CH_2CH(NH_2)CO_2H$ (L)	NH_2	CH	CH
8-aza-SAH	$-SCH_2CH_2CH(NH_2)CO_2H$ (L)	NH_2	N	N
N^6-Me-SAH	$-SCH_2CH_2CH(NH_2)CO_2H$ (L)	$NHCH_3$	N	CH
N^6-Me_2-SAH	$-SCH_2CH_2CH(NH_2)CO_2H$ (L)	$N(CH_3)_2$	N	CH

gel, 1 part (0.01 M phosphate buffer, pH 7.0) and 9 parts (EtOH–HOAc–H_2O, 50:3:5)].

2′,3′-Isopropylidene-L-SAC was hydrolyzed to L-SAC using 0.5 N H_2SO_4 for 48 h at ambient temperature. The reaction mixture was neutralized to pH 5.0 with lead carbonate and filtered and the H_2O removed by lyophilization. The product was crystallized (EtOH–H_2O) to yield 305 mg (40%): mp 215–217° (lit.[8] 223–224°).

***S*-Tubercidinyl-L-homocysteine (STH).** STH had previously been prepared by Coward et al.[10] through the intermediate formation of the 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulfonyl-7-deazaadenosine. We report here the synthesis of STH by a two-step procedure using 5′-chloro-5′-deoxy-7-deazaadenosine. Tubercidin (7-deazaadenosine) (500 mg, 0.53 mmol) was allowed to react with thionyl chloride (1.0 ml) in 5 ml of hexamethylphosphoramide for 19 h using the general conditions previously described.[7] The reaction was quenched with ca. 20 ml of H_2O, concentrated in vacuo to 10 ml, and then neutralized to pH 7 with 2 N aqueous ammonia. The solution was then lyophilized after which the 5′-chloro-5′-deoxy-7-deazaadenosine was crystallized from MeOH–acetone to yield 390 mg (37%): mp 165–167°; NMR (DMSO-d_6) δ 3.87 (m, 2 H, C_5′-H), 4.07 (m, 2 H, C_2′-H and C_3′-H), 4.50 (m, 1 H, C_4′-H), 5.40 (br, 2 H, OH), 6.13 (d, 1H, C_1′-H), 7.00 (br, 2 H, $-NH_2$), 6.65, 7.33 (2 s, 2 H, C_7-H and C_8-H), 8.11 (s, 1 H, C_2-H).

The 5′-chloro-5′-deoxy-7-deazaadenosine (285 mg, 1 mmol) was allowed to react with L-homocystine (268 mg, 1 mmol) in Na and liquid NH_3 according to the general procedure described earlier from our laboratory.[7] The product was purified by ion-exchange chromatography (Dowex 50W-X4, 50–100 mesh, NH_4^+ form) eluting with H_2O followed by 1 N NH_4OH. The appropriate fractions containing product were concentrated in vacuo and further purified by preparative thick-layer chromatography [Avicel F, 1000 μ, EtOH–H_2O (3:2)]. The STH was recovered from the Avicel by elution with H_2O and lyophilization afforded 112 mg (29%): mp 178–180°. (STH was previously prepared by Coward et al.;[10] however, no melting point was reported.) Anal. ($C_{15}H_{21}N_5O_5S$) C, H, N.

***S*-8-Azaadenosyl-L-homocysteine (8-Aza-SAH).** 8-Azaadenosine was prepared according to the procedure of Montgomery et al.[11] by condensation of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (prepared from tetra-*O*-acetyl-D-ribofuranose by room temperature reaction for 3–5 h with HCl gas in Et_2O according to the method of Davoll et al.[12]) with 6-nonamido-8-azapurine.[13] The condensation was run in a benzene slurry in the presence of Linde molecular sieves (AW 500, $^1/_{16}$ in.). The desired 2′,3′,5′-triacetyl-6-nonamido-8-azaadenosine was purified by chromatography on silica gel [EtOAc–$CHCl_3$ (1:1)] and then hydrolyzed with methanolic ammonia to generate the desired 8-azaadenosine. 5′-Chloro-5′-deoxy-8-azaadenosine was prepared quantitatively from 8-azaadenosine using a procedure identical with that described earlier by Montgomery et al.[14]

L-Homocystine (200 mg, 0.75 mmol, 1.4 equiv) was condensed with 5′-chloro-5′-deoxy-8-azaadenosine (300 mg, 1.05 mmol) in Na and liquid ammonia according to the general procedure previously described.[7] After reaction was completed the product was purified by ion-exchange chromatography (Dowex 50W-X4, 50–100 mesh, NH_4^+ form) eluting with H_2O followed by 1 N NH_4OH. The appropriate fractions were concentrated in vacuo and further purified by preparative thick-layer chromatography [Avicel F, 1000 μ, EtOH–H_2O (3:2)]. The 8-aza-SAH was eluted from the Avicel F with H_2O and lyophilized to yield 159 mg (39%): mp 198–199°. Anal. ($C_{13}H_{19}N_7O_5S\cdot H_2O$) C, H, N.

***S*-N^6-Methyladenosyl-L-homocysteine (N^6-Me-SAH).** Using the general procedure described above for the preparation of STH, N^6-methyladenosine (250 mg, 0.89 mmol) was allowed to react with thionyl chloride (0.4 ml) in 3.0 ml of hexamethylphosphoramide for 18 h. The desired 5′-chloro-5′-deoxy-N^6-methyladenosine crystallized after the reaction mixture was neutralized to pH 7.0 with 5% NaOH. The solid which formed was isolated by filtration to yield 240 mg (90%): mp 165–166°. Anal. ($C_{11}H_{14}N_5O_3Cl\cdot H_2O$) C, H, N.

The 5′-chloro-5′-deoxy-N^6-methyladenosine (125 mg, 0.418 mmol) was allowed to react with L-homocystine (70 mg, 0.261 mmol, 1.25 equiv) in Na and liquid ammonia according to the general procedure described previously.[7] The desired N^6-Me-SAH was isolated and purified by ion-exchange chromatography (Dowex 50W-X4, 50–100 mesh, NH_4^+ form) as described above for 8-aza-SAH to yield 76 mg (45%): mp 208–210°. (N^6-Me-SAH was previously prepared by Hildesheim et al.,[15] however, no melting point was reported.)

***S*-N^6-Dimethyladenosyl-L-homocysteine (N^6-Me_2-SAH).** The 5′-chloro-5′-deoxy-N^6-dimethyladenosine was prepared by reaction of N^6-dimethyladenosine (250 mg, 0.847 mmol) with thionyl chloride (0.4 ml) in hexamethylphosphoramide (2.5 ml) for 18 h. The reaction mixture was quenched with 10 ml of H_2O and concentrated in vacuo to ~5 ml. The concentrated reaction mixture was neutralized to pH 8.0 with 2 N NH_4OH, resulting in crystallization of the desired 5′-chloro-5′-deoxy-N^6-dimethyladenosine. The product was recovered by filtration, washed with cold H_2O, and dried to yield 218 mg (82%): mp 150–151°. Anal. ($C_{12}H_{16}N_5O_3Cl\cdot H_2O$) C, H, N.

The 5′-chloro-5′-deoxy-N^6-dimethyladenosine (157 mg, 0.5 mmol) was condensed with L-homocystine (100 mg, 0.374 mmol, 1.5 equiv) in Na and liquid ammonia using a general procedure previously described from our laboratory.[7] The product was purified by ion-exchange chromatography (Dowex 50W-X4, 50–100 mesh, NH_4^+ form) eluting with an ammonia gradient and then by preparative thick-layer chromatography [Avicel F, 1000 μ, EtOH–H_2O (3:2)] as described above in detail for 8-aza-SAH. A yield of 28 mg (14%) of the desired N^6-Me_2-SAH was obtained: mp 70° dec; uv_{max} (H_2O) 275 nm (ϵ 23 500); NMR(D_2O) δ 8.1 and 7.9 (2 s, 2 H, C_2-H, C_8-H), 5.90 (m, 1 H, C_1′-H), 4.3 (m, 3 H, C_2′-H, C_3′-H, C_4′-H), 3.9 (m, 1 H, C_α-H), 3.1 [s, 6 H, $N(CH_3)_2$], 2.8–1.8 (m, 6 H, C_5′-H_2, C_γ-H_2, C_β-H_2).

Enzyme Purification and Assay. The enzymes used in this study were purified from the following sources according to previously described procedures: COMT,[16] rat liver (male, Sprague–Dawley, 180–200 g); PNMT,[17] bovine adrenal medulla

Table I. Inhibition of COMT, PNMT, HMT, HIOMT, and INMT by SAH Analogues[a]

Compd	Inhibitor[b] concn, mM	% inhibition				
		COMT	PNMT	HMT	HIOMT	INMT
L-SAH	0.2	39	49	40	71	65
	2.0	87	92	89	94	95
D-SAH	0.2[c]	5[c]	14[c]	73[c]	1[c]	0[c]
	2.0	22	32	93	6	8
D-SAH sulfoxide	0.2	0	0	0	0	0
	2.0	4	0	0	0	0
N^{α}-Ac-D-SAH	0.2	0	0	0	0	0
	2.0	9	8	0	0	0
L-SAC	0.2	0	11	14	12	0
	2.0	5	27	43	33	2
STH	0.2	56	68	50	24	86
	2.0	94	94	89	79	98
8-Aza-SAH	0.2	5	8	4	49	5
	2.0	28	24	33	89	28
N^{6}-Me-SAH	0.2	15[d]	11[d]	11[d]	7[d]	62
	2.0	55	42	28	13	98
N^{6}-Me_2-SAH	0.2	6	3	0	0	3
	2.0	18	8	4	0	8

[a] The enzymes were purified and assayed as described in the Experimental Section, except in each case the SAM concentration = 1.0 mM. [b] The inhibitors were prepared in aqueous stock solution (10.0 μmol/ml). [c] Taken from ref 4a and 5. [d] Taken from ref 4b.

Table II. Inhibition Constants for Analogues of SAH toward COMT, PNMT, HMT, HIOMT, and INMT[a]

Inhibitors	Inhibition constant, μM,[b] K_{is} ± SEM				
	COMT	PNMT	HMT	HIOMT	INMT
L-SAH	36.3 ± 2.20[c]	29.0 ± 2.84[c]	18.1 ± 2.19[c]	18.5 ± 1.9[c]	8.65 ± 0.71[d]
STH	36.4 ± 3.26	80.5 ± 18.6	17.7 ± 1.56	90.0 ± 9.26	5.26 ± 0.74
8-Aza-SAH	935 ± 122	747 ± 82.4	532 ± 21	18.5 ± 3.68	313 ± 55
N^{6}-Me-SAH	867 ± 112[c]	264 ± 63[c]	1541 ± 205[c]		17.4 ± 5.7

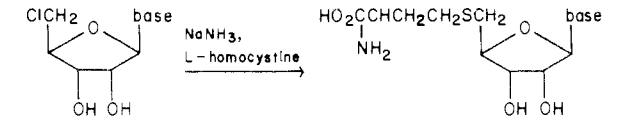
[a] COMT, PNMT, HMT, HIOMT, and INMT were purified and assayed as described in the Experimental Section except SAM concentration = 24–210 μM. [b] Each inhibitor showed linear competitive kinetics.[21] [c] Taken from ref 4b. [d] Taken from ref 5.

(Pel-Freez Biologicals); HMT,[18] guinea pig brain (Pel-Freez Biologicals); HIOMT,[19] bovine pineal glands (Pel-Freez Biologicals); and INMT,[20] rabbit lung (Pel-Freez Biologicals). The enzyme activities were measured and the analogues of SAH evaluated as inhibitors using the radiochemical techniques described in the preceeding papers of this series.[4-6] In the experiments for screening the SAH analogues as enzyme inhibitors (Table I), the assay mixtures contained SAM-$^{14}CH_3$ (0.05 μCi, 1.0 mM) and the appropriate acceptor molecules with their final concentrations being as follows: COMT, 3,4-dihydroxybenzoate (2.0 mM); PNMT, DL-β-phenylethanolamine (1.0 mM); HMT, histamine (1.0 mM); HIOMT, *N*-acetylserotonin (1.0 mM); and INMT, *N*-methylserotonin (1.0 mM). In the kinetic experiments for determining inhibition constants (Table II), the concentrations of the acceptor substrates were held constant at the levels mentioned above, but the SAM concentrations were varied from 24 to 210 μM. The inhibition constants were calculated according to the method of Cleland[21] using a Hewlett-Packard 2100A digital computer and a FORTRAN IV program.

Results and Discussion

Chemistry. The amino acid and base-modified analogues prepared in this study (Chart I) and their synthetic intermediates were characterized by their ir, NMR, and uv spectral properties, their chromatographic properties, and elemental analyses. The N^{α}-Ac-D-SAH was prepared by direct acetylation of D-SAH[7] using Ac_2O. L-SAC, which had been prepared previously by Hildesheim et al.[8] through the intermediate 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine, was prepared in this study by direct condensation of L-cystine with 5′-chloro-5′-deoxyadenosine in Na and liquid ammonia. This overall two-step procedure for the preparation of L-SAC from adenosine provided better overall yields than the previously described method.[8] The base-modified analogues of SAH (STH, 8-aza-SAH, N^6-Me-SAH, and N^6-Me_2-SAH)

Scheme I



base = 7-deazaadenine, 8-azaadenine, N^6-methyladenine, N^6-dimethyladenine

were also prepared by the condensation of the appropriate 5′-chloro-5′-deoxy nucleoside with L-homocystine in Na and liquid ammonia (Scheme I). These 5′-chloro-5′-deoxynucleosides were, in turn, prepared from the appropriate nucleosides and thionyl chloride in hexamethylphosphoramide.[7,9] This two-step synthetic procedure used to prepare SAH analogues[7] has many advantages over the other procedure which uses the 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulfonyl nucleosides as the intermediates.[4b]

Enzyme Inhibitor Studies. In earlier studies from our laboratory, we have investigated the inhibitory activity of various amino acid modified analogues of SAH.[4a,5] In general, it was concluded that the terminal amino group, the terminal carboxyl group, the sulfur atom, and the L configuration of the asymmetric amino acid carbon were required for maximum binding of SAH. However, in these studies[4a] we did detect one interesting exception to these general requirements—that was the very potent and specific inhibitory effect of D-SAH on HMT. In fact, D-SAH (K_{is} = 10.5 ± 1.3 μM) was found to be a more potent inhibitor of HMT than was L-SAH (K_{is} = 18.1 ± 2.19 μM). Since we had shown earlier that *S*-adenosylthiopropylamine (decarboxylated SAH), *S*-adenosyl-4-thiobutyric acid (deaminated SAH), N^{α}-Ac-L-SAH, L-SAH sulfoxide, and L-SAH sulfone were essentially devoid of

inhibitory activity with HMT, it would appear that HMT binding of L-SAH involved at least three points of attachment on the homocysteine position.[4a] Therefore, our original hypothesis[4a] explaining the strong binding of D-SAH to HMT was the existence of alternative attachment sites for either the terminal amino or terminal carboxyl group in order to maintain the optimal three points of attachment. To further substantiate this earlier hypothesis, we have prepared N^{α}-Ac-D-SAH and D-SAH sulfoxide in this study so as to evaluate whether the terminal amino group and the sulfur atom are required for the binding of D-SAH to HMT and whether the basic structure–activity relationships for D-SAH are consistent with those seen with L-SAH. Shown in Table I are the inhibitory activities of N^{α}-Ac-D-SAH and D-SAH sulfoxide toward COMT, PNMT, HMT, HIOMT, and INMT. As would be expected, both N^{α}-Ac-D-SAH and D-SAH sulfoxide were completely devoid of inhibitory activity toward COMT, PNMT, HIOMT, and INMT. The inactivity of these compounds results not only because of the D configuration at the asymmetric amino acid carbon but also because of the alterations in the terminal amino group or sulfur atom, both of which have been shown to be essential for binding to these enzymes in earlier studies.[4a] In addition, the fact that both N^{α}-Ac-D-SAH and D-SAH sulfoxide were also inactive as inhibitors of HMT proves that D-SAH binds to HMT via the same functional groups as does L-SAH. The only difference appears to be that the D configuration of the asymmetric amino acid carbon offers a more favorable arrangement of the functional groups than those of L-SAH to the HMT binding site.

A structural feature of the homocysteine portion of SAH not explored in our earlier studies[4a] is the distance requirement between the carbon atom containing the terminal functional groups and the sulfur atom. Hildesheim et al.[8,15,22,23] have previously reported moderate inhibitory activity of S-adenosyl-L-cysteine, a homologue of L-SAH, toward N-(2-guanine) methyltransferase. We have prepared L-SAC in this study and examined its activity as an inhibitor of COMT, PNMT, HMT, HIOMT, and INMT. The results are shown in Table I. For comparison, we have also listed the inhibitory data for L-SAH toward these enzymes. From the poor inhibitory activity shown in Table I for L-SAC, it is apparent that removal of one carbon atom from the amino acid side chain of L-SAH greatly reduces its ability to bind to these enzymes. The poor inhibitory activity of L-SAC suggests that the three points of attachment on the homocysteine portion of the SAH (the terminal amino group, the carboxyl groups, and the sulfur atom) have rather rigid spacial requirements in order to maintain optimal fit on the enzyme surface.

The various base-modified analogues of SAH (STH, 8-aza-SAH, N^6-Me-SAH, and N^6-Me_2-SAH, which were synthesized as parts of this study, were also tested as inhibitors of COMT, PNMT, HMT, HIOMT, and INMT, and the results are shown in Tables I and II. Included for comparison in Table II are the inhibition constants for L-SAH toward these enzymes. For analogues showing inhibitory activity in the preliminary experiments (Table I), kinetic experiments were carried out and inhibition constants were calculated (K_{is}) (Table II). In all cases competitive kinetic patterns were observed when SAM was the variable substrate.

Results from earlier studies[4b,5] with SAH analogues having modifications in the 6-amino group of adenine suggested that this group contributes significantly to the binding of SAH at enzymatic sites. However, one apparent exception to this general requirement was INMT, which

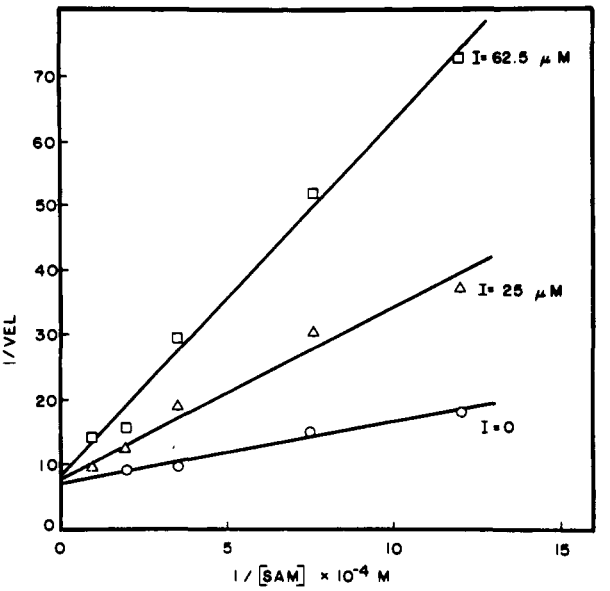


Figure 1. N^6-Me-SAH inhibition of INMT. Plots of reciprocal velocity vs. reciprocal SAM concentrations in the presence of various amounts of N^6-Me-SAH. Assay conditions are outlined in the Experimental Section except SAM concentration = 3.3–53 μM. N-Methylserotonin concentration, 1.0 mM. Vel = nmol of product/mg of protein/min.

was inhibited significantly by N^6-methyl-3-deazaadenosyl-L-homocysteine (N^6-Me-3-deaza-SAH),[5] suggesting that this enzyme could tolerate changes in the 6-amino group of adenine without adversely affecting enzymatic binding. Therefore, we have examined the inhibitory activities of N^6-Me-SAH and N^6-Me_2-SAH toward this enzyme. In earlier studies[4b] we had reported that N^6-Me-SAH was devoid of inhibitory activity toward HIOMT and it had only weak activity toward COMT, PNMT, and HMT. These results are provided in Tables I and II for comparison sake. Consistent with the activity we had observed earlier with N^6-Me-3-deaza-SAH,[5] we have found N^6-Me-SAH to be a very potent competitive inhibitor of INMT (Figure 1). The fact that N^6-Me-SAH is inactive or only weakly active toward the other enzymes tested suggests that this SAH analogue is a potentially selective inhibitor of INMT.

The corresponding N^6-Me_2-SAH was observed to be completely devoid of inhibitory activity toward all of the enzymes tested including INMT. With COMT, PNMT, HMT, and HIOMT these results are consistent with the data observed for the analogues in the 3-deaza-SAH series.[4b] Since it would be expected that pK_a values for the 6-amino group of SAH, the 6-methylamino group of N^6-Me-SAH, and the 6-dimethylamino group of N^6-Me_2-SAH would be similar, the differences in biological activity must result because of increased steric bulk rather than a change in pK_a values.

Since some interesting differences in inhibitory activities were previously observed with SAH analogues containing modified adenine bases (e.g., 3-deaza-SAH), we have prepared and studied several other base-modified analogues of SAH. Shown in Tables I and II are the inhibitory activities of STH (7-deazaadenine replacing adenine) and 8-aza-SAH (8-azaadenine replacing adenine). Coward et al.[10] had previously reported the synthesis of STH and its activity as an inhibitor of COMT, INMT, and tRNA methyltransferase. Consistent with their results,[10] we have also found STH to be a potent inhibitor of COMT and INMT. However, in addition we observed that this

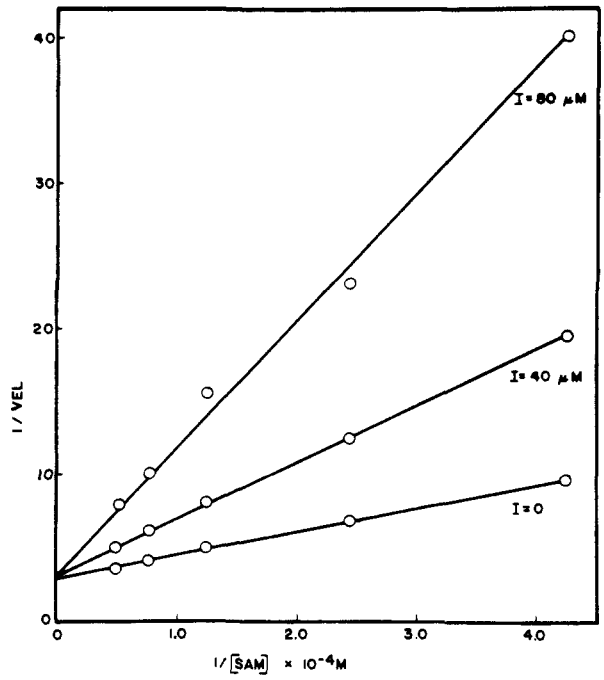


Figure 2. 8-Aza-SAH inhibition of HIOMT. Plots of reciprocal velocity vs. reciprocal SAM concentration in the presence of various amounts of 8-aza-SAH. Assay conditions are outlined in the Experimental Section except SAM concentration = 24–210 μM. *N*-Acetylserotonin concentration, 1.0 mM. Vel = nmol of product/mg of protein/min.

analogue was not quite as active as SAH, but still a fairly potent inhibitor of PNMT and HIOMT. STH also had inhibitory activity comparable to SAH toward HMT. Therefore, in general, it appears that replacement of the adenine of SAH by 7-deazaadenine (STH) does not adversely effect enzymatic binding. As a result, STH is a potent inhibitor of all the methyltransferases tested and, therefore, its potential usefulness as an inhibitor of specific methyltransferases in vivo is limited, because of its general inhibitory activities toward most enzymes in this class.

In contrast to STH, however, 8-aza-SAH exhibited an interesting and somewhat exciting inhibitory profile as shown in Tables I and II. 8-Aza-SAH was shown to have very weak inhibitory activity toward COMT, PNMT, HMT, and INMT but very strong inhibitory activity toward HIOMT. The kinetic pattern for inhibition of HIOMT by 8-aza-SAH was determined to be competitive when SAM was the variable substrate (Figure 2). The inhibition constant of 8-aza-SAH (K_{is} = 18.5 ± 3.68 μM) toward HIOMT is identical with SAH itself (K_{is} = 18.5 ± 1.9 μM). In contrast, with COMT, PNMT, HMT, and INMT, 8-aza-SAH has inhibition constants 20–40 times greater than SAH. This very selective activity of 8-aza-SAH toward HIOMT was extremely interesting, because this enzyme appears to have the strictest specificity for the structural features of SAH. All of the amino acid, base, and sugar modified SAH analogues prepared earlier in our laboratory were essentially inactive as inhibitors of this enzyme.[4] Therefore, 8-aza-SAH is the only potent but yet specific inhibitor of HIOMT uncovered in these studies.

Conclusion

In this study and earlier studies,[4-6] we have attempted to determine the differences in the structural features of SAH which are needed to produce maximal binding to enzymes which require SAM as a methyl donor. In our earlier studies,[4,5] we have demonstrated that the enzymes COMT, PNMT, HMT, HIOMT, and INMT exhibit rather strict specificity for the structural features of SAH. This specificity was determined by synthesizing numerous amino acid, base, or sugar modified analogues of SAH and evaluating these as enzyme inhibitors. However, interesting differences in the specificity of these enzymes for SAH analogues have been detected which may provide the information needed to design in vivo inhibitors specific for the individual members of this large class of enzymes. For example, in an earlier study,[4a] we observed that D-SAH was a potent and fairly specific inhibitor of HMT. D-SAH showed no inhibitory activity toward COMT, PNMT, HIOMT, and INMT. Earlier work by Hildesheim et al.[15] had shown that D-SAH was also a potent inhibitor of *Escherichia coli* tRNA methyltransferase. Therefore, it would appear that HMT and tRNA methyltransferases can tolerate changes in at least the configuration of the asymmetric amino acid carbon of SAH, whereas other methyltransferases cannot.

In contrast, it was observed that PNMT can tolerate changes in the structure of the sugar portion of SAH,[4c,6] whereas other enzymes have high specificity for this portion of the molecule. For example, 3′-deoxyadenosyl-L-homocysteine and *S*-aristeromycinyl-L-homocysteine were found to be rather specific and potent inhibitors of PNMT.[4c,6] These data would suggest that these sugar-modified SAH analogues,[4c,6] and other sugar-modified SAH analogues yet to be identified, may be useful in vivo inhibitors of PNMT. This possibility is being further explored in our laboratory.

Other differences in the specificity for the binding of SAH have been uncovered in this study. For example, it was shown that INMT is capable of tolerating changes in the 6-amino group of SAH, whereas COMT, PNMT, HMT, and HIOMT are not. We arrived at this conclusion because N^6-Me-SAH and N^6-Me-3-deaza-SAH were found to be very potent inhibitors of INMT but not inhibitors of the other enzymes tested. These observations would suggest that greater specificity for INMT may be attainable in an inhibitor by appropriate changes in the nature of the alkyl substituent in the 6-amino group of SAH.

The enzyme HIOMT, which was shown in our earlier studies to have a rather strict specificity for the structural features of SAH,[4,6] appears capable of tolerating changes in the 8 position of the adenine portion of SAH, whereas other methyltransferases are not. The fact that 8-aza-SAH was found in these studies to be a specific and potent inhibitor of HIOMT is extremely interesting, because 8-aza-SAH may then be a useful in vivo inhibitor of HIOMT. In addition, however, it has provided the necessary information which will lead to the synthesis of other SAH analogues modified in the 8 position, which might be useful as inhibitors of this enzyme in vivo.

The results obtained in this study and those reported earlier from our laboratory[4-6] would suggest that differences do exist in the requirements for binding of SAH to methyltransferases and that appropriately modified SAH analogues may be useful inhibitors of specific methyltransferases.

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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-SAM-$^{14}CH_3$, S-adenosyl-L-methionine-*methyl*-^{14}C; L-SAH, S-adenosyl-L-homocysteine; STH, S-tubercidinyl-L-homocysteine; 8-aza-SAH, S-8-azaadenosyl-L-homocysteine; N^6-Me-SAH, S-N^6-methyladenosyl-L-homocysteine; N^6-Me_2-SAH, S-N^6-dimethyladenosyl-L-homocysteine; L-SAC, S-adenosyl-L-cysteine; D-SAH, S-adenosyl-D-homocysteine; D-SAH sulfoxide, S-adenosyl-D-homocysteine sulfoxide; N^α-Ac-D-SAH, S-adenosyl-D-N-acetylhomocysteine; 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); INMT, indoleethylamine N-methyltransferase; K_{is}, inhibition constant for the slope.
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Potential Inhibitors of S-Adenosylmethionine-Dependent Methyltransferases. 5. Role of the Asymmetric Sulfonium Pole in the Enzymatic Binding of S-Adenosyl-L-methionine

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The configuration at the asymmetric sulfonium pole of S-adenosyl-L-methionine (SAM) necessary for optimal enzymatic binding and methyl donation has been elucidated in this study. For the transmethylations catalyzed by catechol O-methyltransferase, phenylethanolamine N-methyltransferase, histamine N-methyltransferase, and hydroxyindole O-methyltransferase, it was demonstrated that only the natural (-) enantiomer of SAM was active as a methyl donor. The corresponding (+)-SAM, which was prepared by enzymatic resolution of synthetic (±)-SAM, was shown to be inactive as a methyl donor in these enzymatic reactions. The (+)-SAM was found, however, to be a potent inhibitor of each of these enzyme-catalyzed transmethylations. These results suggest that the (+) enantiomer offers a nonproductive configuration for the methyl-transfer reaction itself; however, this configuration fails to hamper enzymatic binding. These results are discussed relative to the geometric requirements necessary for the methyl-transfer reaction and the requirements for enzymatic binding.

For numerous biological transmethylation reactions, the natural methyl donor is S-adenosylmethionine (SAM).[2] Many of the structural features of the amino acid, sugar, and base portions of SAM which are required to produce optimal enzymatic binding and maximal rates of methyl transfer have been elucidated in an accompanying paper in this series.[3] The functional group of fundamental importance in the transmethylation reaction itself is the sulfonium pole. Modifications of the sulfonium center of SAM have primarily involved the replacement of sulfur by selenium[4] and the replacement of the methyl group by an ethyl[4b,5] or by an *n*-propyl[6] group. In addition, earlier studies[7-11] have investigated the role of the configuration of the sulfonium pole in these enzyme-catalyzed transmethylations. Because the absolute configuration of the sulfonium center has not yet been determined, stereoisomers are designated (+) and (-) based on polarimetry. De La Haba et al.[7] have shown that SAM prepared enzymatically using adenosine triphosphate:L-methionine S-adenosyltransferase (E.C. 2.5.1.6) has the (-) sulfonium configuration and, therefore, is referred to as (-)-L-SAM. SAM chemically synthesized from the methylation of S-adenosyl-L-homocysteine[7] or by condensation of 5'-methylthioadenosine with 2-amino-4-bromobutyric acid[8] is racemic at the sulfonium pole and is referred to as (±)-L-SAM. SAM with the (+) sulfonium configuration [(+)-L-SAM] has been prepared by treatment of (±)-L-SAM with guanidinoacetate methyltransferase (E.C. 2.1.1.2), which selectively utilizes only the (-)-L-SAM as a substrate.[7,10] By using these purified stereoisomers of SAM [(-)-L-SAM, (+)-L-SAM, and (±)-L-SAM], it has been demonstrated that most methyltransferases show a high degree of specificity for the (-) sulfonium configuration in the methyl-transfer reaction itself; e.g., only the (-)-L-SAM was shown to be a substrate for histamine N-methyltransferase (HMT),[11] hydroxyindole O-methyltransferase (HIOMT),[11] catechol O-methyltransferase (COMT),[7] guanidinoacetate methyltransferase,[7,11] and S-adenosylmethionine decarboxylase.[11] With each of these enzymes the corresponding (+)-L-SAM was not a methyl