Specificity of Inhibition of Adenosine Deaminase by Trialcohols Derived from Nucleosides†

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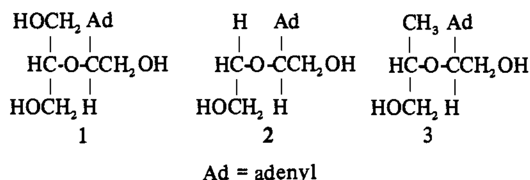
Trialcohols derived from 8-bromoadenosine (5), inosine (7), 6-mercapto-9-β-D-ribofuranosylpurine (10), and 2-amino-9-β-D-ribofuranosylpurine (16) were prepared and found not to be inhibitors of adenosine deaminase. The trialcohols 5, 7, and 16 were prepared by periodate oxidation of the ribofuranose rings followed by reduction of the dialdehydes with sodium borohydride. The trialcohol 10 was prepared from 7 by benzoylation of the hydroxyl groups of the latter, reaction with phosphorus pentasulfide, and base-catalyzed removal of the benzoyl groups. It was concluded from the lack of enzymatic activity of these trialcohols that the 6-amino group was necessary for inhibitory activity and that inhibitors in this group were bound to different sites on adenosine deaminase than were nucleosides which were substrates or inhibitors. Compound 5 was not a substrate for the enzyme.

Adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) is an enzyme which catalyzes the hydrolytic degradation of adenosine to inosine and ammonia. This enzyme is also of chemotherapeutic importance for it causes the deamination of some purine nucleoside analogs, compounds which have a potential usefulness as antitumor agents. Because of this, a number of studies have been reported in recent years in which structure-activity relationships have been described and from which many of the structural requirements necessary for substrate activity or inhibitory activity have been derived.[1-5] Much of this work has been accomplished using adenosine deaminase from calf intestinal mucosa, an enzyme that is commercially available.

In a prior publication, the authors have reported the inhibition of calf intestinal adenosine deaminase by alcohols derived from adenine nucleosides.[6] The results of this work supported the conclusions of Schaeffer and coworkers who had studied the inhibitory properties of 9-hydroxyalkylpurines.[7-10] The structures of three of these alcohols are shown in Chart I as Fischer projection formulas and it

Chart I



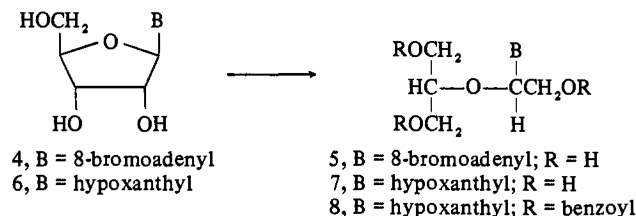
should be noted that each of the alcohols 1-3 can be prepared from more than one nucleoside. In each case, the nucleoside precursor could be a substrate, an inhibitor, or simply an inactive compound. Since the inhibitory properties of the alcohols derived from adenine nucleosides, as well as the 9-hydroxyalkyladenines, could be related to binding sites other than those required for either substrate or inhibitory activity of the nucleosides themselves, it became of interest to prepare alcohols from nucleosides having a purine other than adenine, in order to gain further insight into binding requirements at the purine ring in this class of compounds. For this reason, we decided to prepare trialcohols from a nucleoside known to be a strong inhibitor of adenosine deaminase (2-amino-9-β-D-ribofuranosylpurine), two nucleosides known to be weak inhibitors (inosine and 6-mercapto-9-β-D-ribofuranosylpurine), and a nucleoside having no activity (8-bromoadenosine).

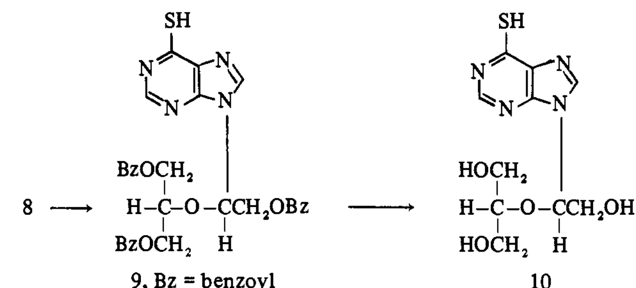
†Part of this work was abstracted from the thesis submitted by R. Rossi in partial fulfillment of the requirements for the Ph.D. degree.

Results

Preparation of Trialcohols Derived from Nucleosides. The general procedure for the preparation of the trialcohols 5, 7, 10, and 16 from preformed nucleosides was the same as reported earlier.[6,11] The sugar rings were oxidized with $NaIO_4$ and the resulting dialdehydes were reduced to the trialcohols with $NaBH_4$. In this manner, trialcohols 5 and 7 were prepared from 8-bromoadenosine (4) and inosine (6), respectively, as crystalline compounds after removal of salts (Scheme I). Compound 5 required further purification

Scheme I





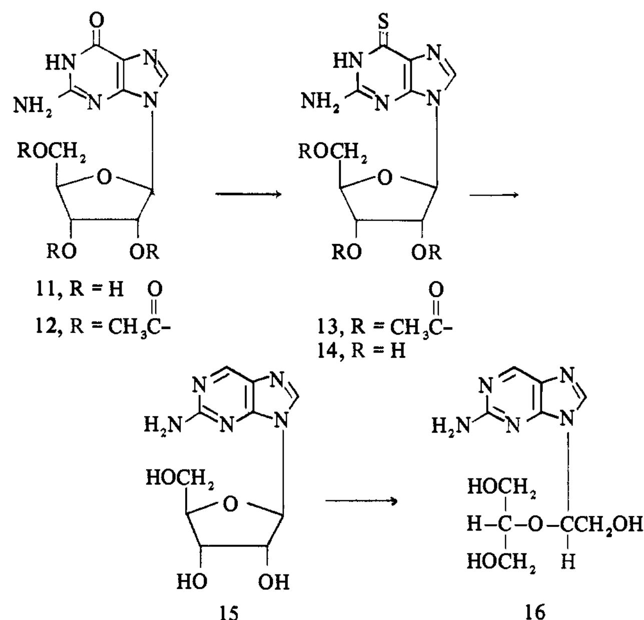
as a picrate before it crystallized as an analytically pure substance.

Because of its accessibility, 7 was used as a starting substance for the synthesis of 10, which can be considered to be the trialcohol derived from 6-mercapto-9-β-D-ribofuranosylpurine (6-MPR). The series of transformations used were based upon a similar synthesis used to convert 6 into 6-MPR.[12] Treatment of 7 with benzoyl chloride in pyridine gave the crystalline tri-*O*-benzoate (8) which was allowed to react with phosphorus pentasulfide in moist pyridine. This reaction mixture had to be kept turbid by occasional addition of water; otherwise 8 was recovered instead of the desired product 9. Fox, *et al.*,[12] have discussed this point previously during their description of the synthesis of certain purine thiols. Removal of the benzoate groups with methanolic sodium methoxide afforded 10.

The preparation of trialcohol 16 was accomplished di-

rectly from 2-amino-9-β-D-ribofuranosylpurine (**15**) in a manner similar to that used above except that it was necessary to use cellulose chromatography in order to purify the product to the extent that crystallization was possible. A simple and rapid synthesis of **15** from guanosine (**11**) was worked out which employed the general scheme of Fox, *et al.*,[12] but which utilized acetate blocking groups instead of benzoate groups (Scheme II). In our hands, the preparation

Scheme II



of tri-*O*-benzoylguanosine was tedious and the purification was difficult, whereas the tri-*O*-acetate **12** was rapidly prepared in good yield, crystallized directly from the reaction mixture, and was suitable for further use at this point. Conversion of **12** to **13** was achieved in the same manner as the conversion of **8** to **9**. Previous preparations of **13** have employed acetylation of thioguanosine (**14**)[13,14] or reaction of 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine with thiourea.[15] Removal of the acetyl groups of **13** gave **14** which was converted to **15** with Raney nickel. In addition to the physical properties of **15**, which established its identity, **15** was found to be strongly inhibitory to adenosine deaminase, as reported by Simon, *et al.*[5]

The structures of **5**, **7**, **10**, and **16** were supported by elementary analyses, spectra, and the known course of the reactions used. The uv and ir spectra of the alcohols had peaks similar to that of the parent nucleosides. The mobilities of the alcohols on paper and their ultraviolet maxima are given in Table I.

Effects of the Alcohols on Adenosine Deaminase. The alcohols **5**, **7**, **10**, and **16** were not inhibitors of adenosine deaminase from calf intestinal mucosa at concentrations up to *ca.* $8 \times 10^{-5} M$. At a similar concentration, **1** gave about 20% inhibition.

Discussion

In our previous article,[6] we demonstrated that inhibitory binding sites on adenosine deaminase could be located which were different from the substrate binding sites. Most substrates were characterized by a cyclic sugar ring, whereas the inhibitors which were derived from nucleosides were essentially branched-chain alcohols. The preferred configuration at the carbon atom which was originally the ano-

Table I. Paper Chromatographic and Ultraviolet Spectral Data of Trialcohols Derived from Nucleosides

Trialcohol	Paper chromatography[a] A	Paper chromatography[a] B	Ultraviolet maxima[b] nm	Ultraviolet maxima[b] $\epsilon \times 10^{-3}$
5	1.62	0.91	264	18.4
7	2.25	0.36	253	13.0
10	2.03	0.59	314	20.0
16	1.89	0.59	304	6.8
			243	5.8

[a]Paper chromatography was performed on Whatman No. 1 paper by a descending technique. Values are reported as Rad which corresponds to the ratio of the distance that the trialcohol moved in comparison to adenine (Rad = 1.00). Solvents: A, 5% aqueous disodium hydrogen phosphate; B, 1-butanol-water (86:14 v/v). Spots were located with an ultraviolet lamp. [b]pH 7.6.

meric carbon atom was found to be *R*. An hydroxyl binding region near N-9 and a distant methyl binding region provided these compounds with two distinct sites at which to bind to the enzyme. Each of these regions could be individually used, but the best inhibition occurred when both of these regions were used simultaneously. Moreover, the hydroxyl binding region was not the same one that was required for substrate activity. These findings were in agreement with studies reported by Schaeffer and coworkers ers.[7,8,16,17] It now became of interest to gain further insight into the inhibitory process, especially as concerned the purine moiety of these alcohols. To do this, several new alcohols having the *R* configuration were prepared from nucleosides in which adenine was not the purine base. Using nucleosides known to be inhibitors of calf intestinal adenosine deaminase, the trialcohols of these compounds (**7**, **10**, **16**) were prepared and found not to inhibit the enzyme. It is important to note that except for **5**, all of these compounds lacked an amino group at position 6 of the purine ring. It appears that for compounds not having a sugar ring and which inhibit by the binding process mentioned above, that this amino group is a requirement for inhibitory activity. In the earlier studies by Schaeffer and coworkers, a variety of 9-hydroxyalkylpurines derived from hypoxanthine and 6-mercaptopurine was also found to be noninhibitory. It must be concluded, therefore, that nucleoside inhibitors bind to adenosine deaminase in a manner different than the alcohols, since a 6-amino group is not required in these cases for inhibitory activity; in fact, no functional group is required on the purine ring at all as exemplified by the activity of 9-β-D-ribofuranosylpurine.[1,4] Chassy and Suhadolnik[3] have pointed out that the substrate binding site may be more specific for the 9 position substituent than for the 6 position, but it is obvious from our studies and that of Schaeffer's group that the inhibitor site is not. It could be that the failure of the trialcohols to bind is not related to the absence of the amino group as such but to the effect that various substituents may have on the basicity of the purine ring nitrogens. The trialcohol derived from 8-bromoadenosine (**5**), on the other hand, does have the 6-amino group and has a structure almost identical with the trialcohol derived from adenosine, which is an inhibitor of adenosine deaminase.[6] However, **5** is neither a substrate nor an inhibitor and the same is true for 8-bromoadenosine itself. It has been proposed that the bromine atom at position 8 causes either a steric interaction or an inductive effect which would account for this behavior.[5]

Because of interest in the potential chemotherapeutic usefulness of compounds related to 6-mercaptopurine or its

nucleosides, **10** will be investigated further as a possible antimetabolite.

Experimental Section

General Materials and Methods. General methods of procedure and instrumentation were described in the previous article.[6] Guanosine and 8-bromoadenosine were purchased from Aldrich Chemical Co. and inosine was purchased from P. L. Biochemicals. Evaporations were performed under reduced pressure in a rotary evaporator with a bath temperature of 40–45°. Elementary analyses were performed by the Baron Consulting Co., Orange, Conn., or by the Spang Microanalytical Laboratory, Ann Arbor, Mich. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

2-*O*-[1(*R*)-[9-(8-Bromoadenyl)]-2-(hydroxy)ethyl]glycerol (5). A suspension containing 3.64 g (10 mmol) of 8-bromoadenosine (**4**) in 75 ml of H_2O was treated with 2.14 g (10 mmol) of $NaIO_4$ in small portions while maintaining the temperature below 20° with an ice bath. The mixture was stirred at room temperature overnight, protected from light. The solution was poured into 150 ml of EtOH, stirred for 20 min, and filtered. The filtrate was evaporated; the residue was dissolved in H_2O and added dropwise to 2 g of $NaBH_4$ in 50 ml of H_2O. After 2 hr the alkaline solution was neutralized with Bio-Rad AG50-X8 (H^+) resin and evaporated to a syrupy residue. Absolute MeOH was evaporated several times to remove boric acid; the syrup was dissolved in 50 ml of MeOH and treated with 100 ml of 10% methanolic picric acid. The flask was chilled in ice for 1 hr and the picrate was filtered off, yielding 4.2 g (72%). Recrystallization from MeOH afforded bright yellow needles which decomposed above 170°, $[\alpha]^{23}D$ +37.1° (*c* 1.51, DMF). *Anal.* ($C_{16}H_{17}BrN_8O_{11}$) C, H, N, Br.

The picrate (3.9 g) was dissolved in 1.6 l. of H_2O and the yellow color was discharged with Bio-Rad AG2-X8 (CO_3^{2-}) resin. The syrup obtained after evaporation was crystallized from a small amount of water after standing overnight in the refrigerator, giving 1.46 g (62%). An analytical sample was obtained by recrystallization: mp 198–201°, with decomposition beginning at 188°; $[\alpha]^{23}D$ +38.0° (*c* 2.03, H_2O). *Anal.* ($C_{10}H_{14}BrN_5O_4$) C, H, N, Br.

2-*O*-[1(*R*)-(9-Hypoxanthyl)-2-(hydroxy)ethyl]glycerol (7). Inosine (**6**) (2.7 g, 10 mmol) was treated with $NaIO_4$ and $NaBH_4$ as described for **4**. After removal of boric acid as methyl borate, a white solid remained which was crystallized from EtOH, 1.73 g (64%). Compound **7** melted at 207°, resolidified, and slowly decomposed above 240°, $[\alpha]^{25}D$ +70.2° (*c* 1.61, H_2O). *Anal.* ($C_{10}H_{14}N_4O_5$) C, H, N.

Tri-*O*-benzoate of 7 (8). A suspension of **7** (1.3 g, 5 mmol) in 200 ml of dry pyridine was stirred with 25 ml of benzoyl chloride for 24 hr at room temperature. The solution was poured into a mixture of saturated $NaHCO_3$ and ice, stirred for 20 min, and extracted with 150 ml of $CHCl_3$. The $CHCl_3$ layer was washed three times with $NaHCO_3$, twice with H_2O, and dried ($MgSO_4$). An orange syrup was obtained after evaporation which was coevaporated with toluene several times to remove traces of pyridine. A solid (2.3 g, 77%) was obtained which was recrystallized from EtOH, mp 179–180°. *Anal.* ($C_{31}H_{26}N_4O_8$) C, H, N.

2-*O*-[1(*R*)-[9-(6-Mercaptopurinyl)]-2-(hydroxy)ethyl]glycerol (10). A mixture containing 7 g (12 mmol) of **8**, 45 g of P_2S_5, and 300 ml of pyridine was heated at reflux for 4 hr. H_2O was added intermittently, so that a slight turbidity persisted, until a total of 5 ml of H_2O had been added. The solvent was evaporated at 55°, leaving a thin syrup which was cautiously added to 500 ml of boiling H_2O. The mixture was stirred for 0.5 hr; the solid was isolated by filtration while hot and washed with hot H_2O and finally with EtOH. The product **9** weighed 6.4 g (88%) and was recrystallized from EtOH, mp 228–231°. *Anal.* ($C_{31}H_{26}N_4O_7S$) C, H, N, S.

Tribenzoate **9** (0.51 g, 0.85 mmol) was suspended in 40 ml of absolute MeOH and the mixture was heated at reflux for 4.5 hr, while the pH was maintained at 9 by intermittent addition of 1 *N* methanolic NaOMe. The solution was adjusted to pH 8 with Bio-Rad AG50W-X8 (H^+) resin and filtered, and the solvent was evaporated. The residue was dissolved in H_2O, the aqueous solution was washed with $CHCl_3$ (3 × 50 ml), and the H_2O was evaporated. Crystallization of the residue from aqueous EtOH afforded 148 mg (61%) of **10**, mp 204°, which resolidified and decomposed upon further heating, $[\alpha]^{26}D$ +73° (*c* 0.5, H_2O). *Anal.* ($C_{10}H_{14}N_4O_4S \cdot {}^1/_3H_2O$) C, H, N, S.

2-Amino-9-β-D-ribofuranosylpurine (15). Tri-*O*-acetylguanosine (**12**) was prepared in good yield from guanosine (**11**) following the directions of Bredereck.[18] A reaction mixture containing 16.3 g (39.8 mmol) of **12**, 34 g (0.153 mol) of P_2S_5, and 500 ml of pyridine was heated at reflux for 6 hr with intermittent addition of small amounts of H_2O to keep the mixture turbid, until a total of 2.9 ml had been added. The mixture was concentrated by evaporation to a thin syrup, which was cautiously poured into 600 ml of boiling H_2O. This was concentrated by boiling to a volume of approximately 400 ml and the mixture was allowed to cool. The solid was collected by filtration and washed with water to afford 10.2 g (60%) of tri-*O*-acetylthioguanosine (**13**). A small sample, which was recrystallized from H_2O, softened at 203° and decomposed slowly above 210° until it melted completely with decomposition at 235–240°. *Anal.* ($C_{16}H_{19}N_5O_7S$) C, H, N, S. Three preparations of **13** have been reported with the following melting points: mp 203–205°,[13] mp 250° dec[15] and mp 241–242° dec.[14]

A suspension of **13** (4.0 g, 9.4 mmol) in 170 ml of absolute MeOH was heated at reflux for 2 hr while the pH was maintained near 10 by intermittent addition of 1 *N* methanolic NaOMe. The MeOH was evaporated; the residue was dissolved in 50 ml of H_2O and washed with $CHCl_3$. The aqueous layer was treated with charcoal (heat) and the pH was adjusted to 5 with AcOH. Crystallization of **14** occurred overnight in the refrigerator. Recrystallization from H_2O afforded 2.4 g (84%); mp 230°; uv $\lambda_{max}^{pH 12}$ 252 and 319.5 nm (lit.[12] mp 227°; uv $\lambda_{max}^{pH 12}$ 252 and 319.5 nm).

A solution of **14** (5.16 g, 16.8 mmol) in 350 ml of boiling H_2O was treated with 10 g of Raney nickel. After 2 hr at reflux, the mixture was filtered, and the H_2O was removed by evaporation. The product **15** was obtained from EtOH: 3.3 g (74%); mp 168°; $[\alpha]^{25}D$ −34° (*c* 1.2, H_2O); uv $\lambda_{max}^{pH 1}$ 313, 246 nm; uv $\lambda_{max}^{pH 3.5}$ 306, 244 nm; uv $\lambda_{max}^{pH 7}$ 303, 243 nm (lit.[12] mp 165°; $[\alpha]^{25}D$ −39° (*c* 1.2, H_2O); uv $\lambda_{max}^{pH 1}$ 313, 246 nm; uv $\lambda_{max}^{pH 3.5}$ 308, 243 nm; $\lambda_{max}^{pH 6.8}$ 304, 243 nm).

2-*O*-[1(*R*)-[9-(2-Aminopurinyl)]-2-(hydroxy)ethyl]glycerol (16). To a solution of **15** (1.34 g, 5 mmol) in 100 ml of H_2O, which was chilled in an ice bath, was added $NaIO_4$ (1.17 g, 5 mmol) in small portions. The solution was stirred for 1 hr in the ice bath and then for 40 min at room temperature. The solution was poured into 400 ml of EtOH and the remaining steps were identical with those already described for the preparation of **5** from **4**. A light yellow foam was obtained after the syrup had been dried by several evaporations of anhydrous EtOH. A portion of this foam (540 mg) was slurried with cellulose powder (Whatman CF11) in 87:13 *n*-BuOH–H_2O and this was applied to the top of a cellulose column (36 × 4 cm). The column was eluted with the same solvent mixture and 8-ml fractions were collected and monitored at 254 nm. Fractions 103–175 were combined and the solvents were evaporated. Crystallization from ethanol gave 278 mg: mp 148–149°; $[\alpha]^{26}D$ +34° (*c* 0.5, H_2O). *Anal.* ($C_{10}H_{15}N_5O_4$) C, H, N.

Enzyme Assay. The details for the assay of adenosine deaminase were given in the previous article[6] and were based upon the procedure of Kaplan[19] in which the rate of change in absorbance at 265 nm was measured at 25° in 0.05 *M* phosphate buffer (pH 7.6). The concentration of adenosine was 7.6 × 10^{-5} *M*.

References

(1) J. G. Cory and R. J. Suhadolnik, *Biochemistry*, 4, 1729 (1965).
(2) S. Frederiksen, *Arch. Biochem. Biophys.*, 113, 383 (1966).
(3) B. M. Chassy and R. J. Suhadolnik, *J. Biol Chem.*, 242, 3655 (1967).
(4) R. Wolfenden, J. Kaufman, and J. B. Macon, *Biochemistry*, 8, 2412 (1969).
(5) L. N. Simon, R. J. Bauer, R. L. Tolman, and R. K. Robins, *ibid.*, 9, 573 (1970).
(6) L. M. Lerner and R. R. Rossi, *ibid.*, 11, 2772 (1972).
(7) H. J. Schaeffer and P. S. Bhargava, *ibid.*, 4, 71 (1965).
(8) H. J. Schaeffer, D. Vogel, and R. Vince, *J. Med. Chem.*, 8, 502 (1965).
(9) H. J. Schaeffer and R. Vince, *ibid.*, 10, 689 (1967).
(10) H. J. Schaeffer and C. F. Schwneder, *J. Pharm. Sci.*, 60, 1204 (1971).
(11) L. M. Lerner, *Carbohyd. Res.*, 13, 465 (1970).
(12) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Amer. Chem. Soc.*, 80, 1699 (1958).
(13) L. R. Lewis, R. K. Robins, and C. C. Cheng, *J. Med. Chem.*, 7, 200 (1964).
(14) T. Naito, K. Ueno, and F. Ishikawa, *Chem. Pharm. Bull.*,

12, 951 (1964).
(15) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, 28, 945 (1963).
(16) H. J. Schaeffer and D. Vogel, *J. Med. Chem.*, 8, 507 (1965).
(17) H. J. Schaeffer, S. Gurwara, R. Vince, and S. Bittner, *ibid.*, 14, 367 (1971).
(18) H. Bredereck, *Chem. Ber.*, 80, 401 (1947).
(19) N. O. Kaplan, *Methods Enzymol.*, 2, 473 (1955).

Analogs of *S*-Adenosylhomocysteine as Potential Inhibitors of Biological Transmethylation. Specificity of the *S*-Adenosylhomocysteine Binding Site†

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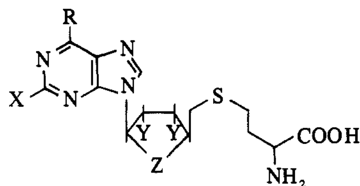
Several structural analogs of *S*-adenosylhomocysteine have been synthesized and their interaction with catechol *O*-methyltransferase (COMT) has been investigated. Kinetic studies on the inhibition of COMT by these compounds have resulted in a delineation of binding forces involved in the inhibition of transmethylation by *S*-adenosylhomocysteine. These data demonstrate a strict specificity in the interaction of *S*-adenosylhomocysteine with COMT. This specificity may be critical in the regulation of biological transmethylation by *S*-adenosylhomocysteine.

The general utilization of *S*-adenosylmethionine (SAM)‡ as a methyl donor in numerous biological reactions has been known for many years.[1] Inhibition by the demethylated product, *S*-adenosylhomocysteine (SAH), seems to be a general feature of many of these enzyme-catalyzed transmethylations.[2] The product inhibition of these methylases presumably is regulated by further breakdown of SAH. It has recently been shown that there is present in the rat brain a hydrolase (*S*-adenosylhomocysteinase) which can degrade SAH and apparently exert a regulatory role in the activity of phenethanolamine *N*-methyltransferase (PNMT).[3] Most studies of the interaction between small molecules and various methylases have been concerned only with the acceptor portion, *e.g.*, catecholamine analogs as inhibitors of PNMT[4] and cathechol *O*-methyltransferase (COMT, E.C. 2.1.1.6).[5,6] More recently, the use of substituted adenosines (cytokinins) as inhibitors of tRNA methylases has been reported.[7] In order to study the nature of the intermolecular forces involved in the binding of SAH to various methylases, we have continued our earlier studies[8] and synthesized a series of compounds with modifications in the nucleoside portion of the SAH molecule (Chart I). Use of these compounds as probes of the COMT-active site permits elucidation of the contribution made by specific moieties of SAH in regulation of methylase activity.

Chart I. Compounds Synthesized to Probe the SAH Binding Site of Catechol *O*-Methyltransferase



Compd	X	Y	Z	R
SAH	H	OH	O	NH_2
1	H	H	CH_2	NH_2
2	H	OH	CH_2	NH_2
3	F	OH	O	NH_2
4	H	OH	O	OH
5	H	$-(CH_2)_5-$		NH_2

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‡Abbreviations used: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; PNMT, phenethanolamine *N*-methyltransferase; COMT, catechol *O*-methyltransferase; SAHO, *S*-adenosylhomocysteine sulfoxide.

Experimental Section

All melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values. Ir spectra were run on a Perkin-Elmer Model 21 spectrophotometer and uv spectra on a Cary Model 15 spectrophotometer. Tlc were run on Eastman chromatograms No. 6060 (silica gel with fluorescent indicator) or No. 6065 (cellulose with fluorescent indicator). Spots were detected by visual examination under uv light and/or with ninhydrin, or PtI_4 spray reagents, for compounds containing amino or thioether moieties, respectively.

COMT was isolated from rat liver and purified as described by Nikodejevic, *et al.*,[6] with minor modifications. Enzyme assays were carried out as described previously;[8,9] substrates and other materials required for the assays were obtained as indicated in the references cited. *S*-Inosylhomocysteine (4) was prepared as described by Zappia, *et al.*[10] Aristeromycin was a gift from Dr. T. Kishi of Takeda Chemical Industries, Ltd. 2-Fluoroadenosine was supplied by Drug Research and Development, Chemotherapy, National Cancer Institute. 9-[*S*-(4-(2-Amino)butyric acid)-5′-thiopentyl]adenine (5) was prepared as previously described.[8] *S*-Adenosylhomocysteine sulfoxide (SAHO) was prepared by the method of Duerre, *et al.*[11]

cis-1′-(6-Amino-9-purinyl)-3′-*S*-cyclopentylmethylhomocysteine (1). *cis*-3-(6-Amino-9-purinyl)cyclopentylcarbinol[12] was prepared by modified literature procedures. This alcohol (1.63 g, 7.02 mmol) was dissolved in 25 ml of dry pyridine, freshly distilled from barium oxide, and the resulting solution cooled to *ca.* 0° in an ice-salt bath. Freshly recrystallized *p*-toluenesulfonyl chloride (1.49 g, 7.89 mmol) was added to the cooled solution in one portion and stirring continued for 5 min. The reaction solution was removed from the ice bath and allowed to stand overnight at ambient temperature. Evaporation of the pyridine *in vacuo* was followed by partitioning the residue between $CHCl_3$ and 3 *N* H_2SO_4 at 4°. The chloroform layer was then washed again with 3 *N* H_2SO_4, H_2O, and finally four times with a saturated solution of $NaHCO_3$. The dried chloroform layer was then concentrated *in vacuo* to give 1.8 g (67%) of an oily residue, sufficiently pure for further transformations. This oily tosylate was not soluble in liquid amonia; therefore it was dissolved in 12 ml of bis(2-ethoxyethyl) ether which had been dried over calcium hydride and distilled from $LiAlH_4$. A solution of homocysteine in liquid NH_3 was generated by adding Na in small pieces (*ca.* 300 mg) to 815 mg (3.6 mmol) of *S*-benzylhomocysteine[13] until a blue color persisted for 15 min. The tosylate solution was added in one portion and the resulting mixture stirred vigorously at −70° for 10 min and allowed to warm slowly to ambient temperature overnight. After evaporation of residual NH_3, the gummy residue was dissolved in 25 ml of H_2O and the pH adjusted to 4 with 1 *N* H_2SO_4. Extraction with $CHCl_3$, followed by lyophilization of the aqueous layer, gave a crude product which could be purified by chromatography on Dowex 1 as described previously for related *S*-alkylhomocysteines.[8] The purified product was a