Multisubstrate Adducts as Potential Inhibitors of S-Adenosylmethionine Dependent Methylases: Inhibition of Indole N-Methyltransferase by (5'-Deoxyadenosyl)[3-(3-indolyl)prop-1-yl]methylsulfonium and (5'-Deoxyadenosyl)[4-(3-indolyl)but-1-yl]methylsulfonium Salts

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Multisubstrate adducts of the indole N-methyltransferase reaction have been designed in which a structural moiety representing the nucleophilic methyl acceptor is attached through the sulfur atom to the 5-(methylthio)adenosine and/or methionine moieties of the methyl donor, S-adenosyl-L-methionine. Indole derivatives attached through a 4-(3-indolyl)butyl sulfide or a 3-(3-indolyl)propyl sulfide linkage to 5'-thioadenosine or homocysteine have been synthesized, together with their corresponding methylsulfonium salts. These compounds have been assayed for their ability to inhibit rabbit lung indole N-methyltransferase. The adenosylsulfonium salts (5'-deoxyadenosyl)[4-(3-indolyl)but-1-yl]methylsulfonium perchlorate and (5'-deoxyadenosyl)[3-(3-indolyl)prop-1-yl]methylsulfonium perchlorate were found to be inhibitors of this enzyme with K_i's of 12 and 44 μM, respectively. Neither of these compounds was effective in inhibiting the methylation of 3,4-dihydroxybenzoic acid, catalyzed by purified porcine catechol O-methyltransferase.

Indole N-methyltransferase (INMT), an S-adenosylmethionine (SAM) dependent enzyme, is present in both human[1-3] and animal tissues[4,5] and catalyzes the conversion of tryptamine to N-methyltryptamine (NMT) and N,N-dimethyltryptamine (DMT). The latter methylation product is a potent psychotomimetic and produces a model psychosis when administered parenterally to normal individuals.[6] The in vivo formation of abnormal amounts of DMT has been implicated in the etiology of schizophrenia and other psychotic conditions,[7] and several clinical studies[8-12] have indicated that blood and urine levels of DMT are elevated in schizophrenic patients when compared to those of normal individuals, while other studies have reported no difference.[13,14] In some cases, the validity of these reports is open to question, due to the ambiguity of the analytical techniques employed.[15,16] We were, therefore, interested in the possibility of developing a selective in vivo inhibitor of tryptamine N-methylation that would not perturb other SAM-dependent methylation reactions, in order to determine the role of tryptamine N-methylation in psychotic disorders.

Several potent in vitro inhibitors of INMT have been reported. S-Adenosylhomocysteine (SAH) is an effective product inhibitor of INMT (K_i = 1.0 × 10^{-5} M),[17] but its nonspecificity[18] and metabolic instability[19] make it unsuitable for in vivo inhibition studies. The metabolically stable synthetic analogue S-tubercidinylhomocysteine (STH)[20,18] shows good inhibitory properties against INMT (K_i = 7 × 10^{-6} M) but lacks specificity for this enzyme.[21] Several synthetic SAH analogues have been investigated for inhibitory properties;[22] of these, N^6-methyl-3-deaza-S-adenosylhomocysteine seems to exert significant inhibition (K_i = 7 × 10^{-5} M) exclusively on INMT, although more studies to determine this are required. The most potent and specific inhibitor of INMT appears to be 2,3,4,6,7,8-hexahydropyrrolo[1,2-a]pyrimidine (DBN).[23] This compound has a K_i of 2 × 10^{-6} M and is a noncompetitive inhibitor with respect to indolamine substrates.

An interesting approach to the design of highly potent and specific enzyme inhibitors involves the use of transition-state analogues,[24,25] whose design is based upon the chemical mechanism of the enzymic reaction. In recent years, a better understanding of the mechanism of methyl group transfer in SAM-dependent enzyme-catalyzed reactions has emerged, which has an important bearing on the design of effective inhibitors of transmethylation reactions. Schowen et al.[26,27] have shown that in the case of the catechol O-methyltransferase (COMT) reaction, the transfer of the methyl group occurs via a tight, symmetrical S_N2 transition state, and Woodard et al.[28] have followed

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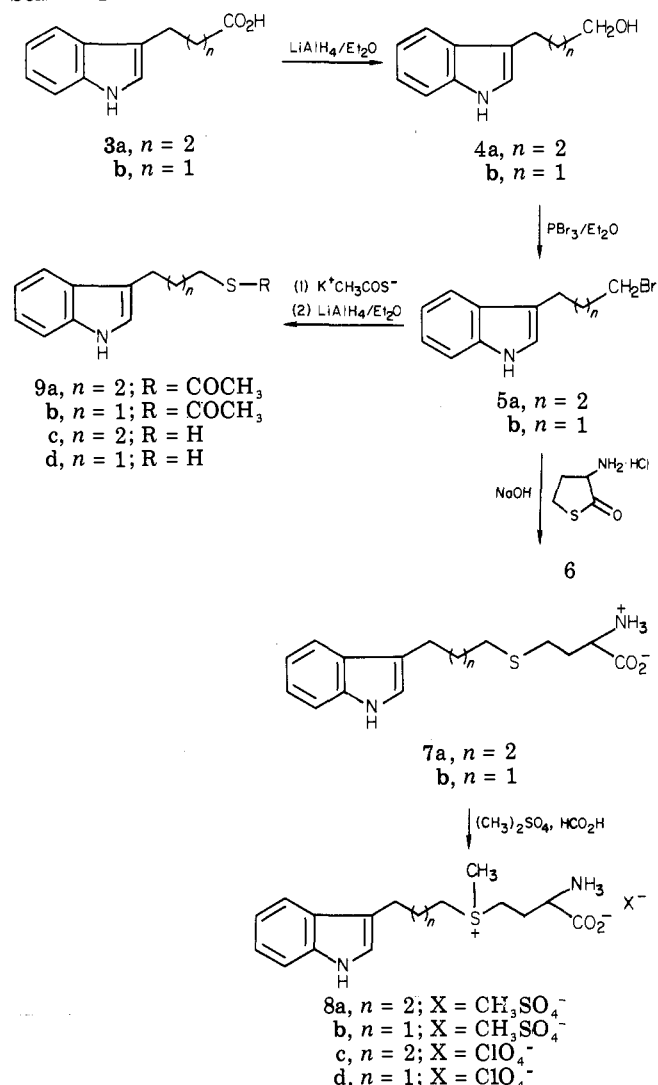(28) R. W. Woodard, M. D. Tsai, H. G. Floss, P. A. Crooks, and J. K. Coward, *J. Biol. Chem.*, 255, 9124 (1980).

the steric course of the methyl group transfer in this reaction, using SAM carrying a methyl group made chiral by labeling with ^{1}H, ^{2}H, and ^{3}H in an asymmetrical arrangement. The results of these latter studies show that transfer of the methyl group occurs in an inversion mode, indicating a direct transfer of the methyl group from the S atom of SAM to the oxygen of the catechol substrate via a classical S_N2 mechanism, without the involvement of a methylated enzyme intermediate. Other studies from Floss's laboratory[29,30] have utilized chirally labeled SAM in other SAM-dependent transmethylation reactions, and these results suggest that transmethylations from SAM to nucleophilic C, N, O, or S atoms in small-molecule substrates involve a direct transfer of the methyl group from the donor to the acceptor molecule.

A reaction mechanism of the "ordered Bi/Bi" type has been suggested for INMT with SAM as the first bound substrate.[17] The proposed transition state for the INMT reaction with tryptamine as substrate is illustrated in structure **1** and represents the point that is midway on the

1

2
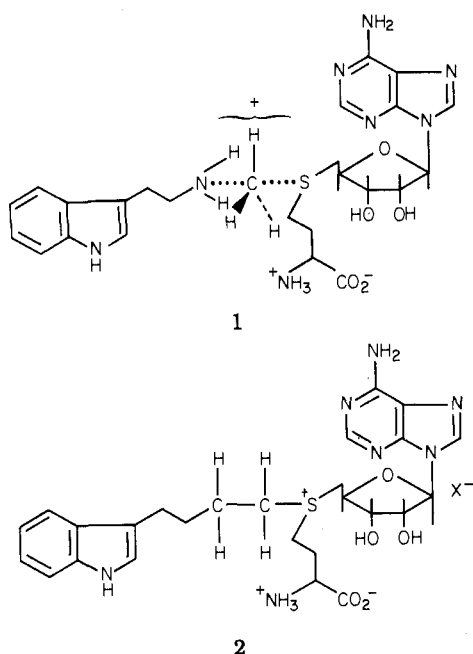
reaction coordinate for the direct nucleophilic attack of the amino group on the methyl group of SAM. This transition state incorporates a colinear arrangement of nucleophile–CH_3–S^+. Structure **2** represents a stable synthetic analogue of this transient state in which the side-chain amino group of tryptamine and the migrating methyl group have been replaced by a CH_2CH_2 group that is covalently linked to the homocysteinyl and adenosyl moieties. This approach to the design of SAM-dependent methylase inhibitors is analogous to that used by Coward et al.,[21,31] who have recently utilized the transition-state analogue approach in the development of mechanism-based multisubstrate adduct inhibitors of the COMT reaction. In this paper, we describe our initial studies on the synthesis of thioether precursors of **2** and their conversion to the methylsulfonium salts **8a,b** and **12a,b**. These compounds lack the third arm, or ligand, around the sulfonium pole and, thus, are incomplete, or biliganded, analogues of the multisubstrate adduct **2**. The results of

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Scheme I

in vitro inhibition of INMT by these compounds are also reported.

Chemistry. The synthetic routes employed for the preparation of the sulfonium salts **8a,b** and **12a,b** are outlined in Schemes I and II. Synthetic routes to structures **7a,b** utilized the appropriate bromo derivative, **5**. In the preparation of **7a**, 3-indolebutyric acid (**3a**) was initially reduced with lithium aluminum hydride in diethyl ether to 3-(3-indolyl)butan-1-ol (**4a**), which could then be converted into the bromo derivative, **5a**, with phosphorous tribromide in diethyl ether. The yield from this latter reaction was only 24%, and the product could only be obtained in pure form after a silica gel chromatographic separation. The preparation of **7a**, by the coupling of **5a** with homocysteine, was patterned on a procedure recently reported by Anderson et al.,[31] which utilizes homocysteine thiolactone hydrochloride (**6**) as a homocysteine precursor. Thus, reaction of **5a** with **6** in aqueous sodium hydroxide at reflux temperature and under a nitrogen atmosphere gave a colorless solution from which **7a** could be precipitated almost quantitatively by treatment of the solution with glacial acetic acid. In a similar manner, 3-indolepropionic acid (**3b**) was reduced to **4b**, followed by bromination to **5b** and reaction of this product with **6** in basic solution to give **7b**.
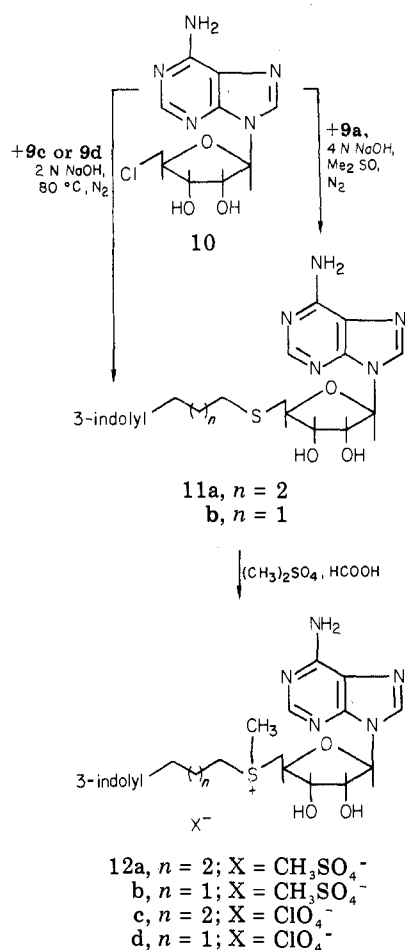
The synthetic route adopted for the preparation of the thioethers **11a,b** was via the corresponding indolyl thiol derivative **9**, using a procedure analogous to that recently

Scheme II

11a, n = 2
b, n = 1

12a, n = 2; X = $CH_3SO_4^-$
  b, n = 1; X = $CH_3SO_4^-$
  c, n = 2; X = ClO_4^-
  d, n = 1; X = ClO_4^-

reported by Benghiat and Crooks[32] for the synthesis of 5'-deoxy-5'-[[(1-methylindol-3-yl)methyl]thio]adenosine. Reaction of 5a with potassium thioacetate in a 1:1 mixture of ethanol and acetone at reflux temperature afforded the thioacetate derivative 9a, which was smoothly reduced to the corresponding thiol, 9c, with lithium aluminum hydride in diethyl ether. The reaction of 9c with 5'-chloro-5'-deoxyadenosine (10) in 2 N aqueous sodium hydroxide at 80 °C under an atmosphere of nitrogen gave 11a in 60% yield. Compound 11a could also be prepared by reaction of 9a with 10 in a mixture of dimethyl sulfoxide and 4 N aqueous sodium hydroxide at room temperature and under an atmosphere of nitrogen. Compound 11b could be prepared from 9b in a manner analogous to the preparation of 11a.

Initial studies on the S-methylation of the thioethers were carried out on 7a with a mixture of iodomethane in formic acid. These conditions have been previously shown to selectively methylate the sulfur atom in 5-deoxy-5-thioadenosine analogues.[31,33] The appearance and gradual increase in intensity of a singlet at δ 3.08 as the reaction of 7a with iodomethane proceeded was indicative of methylsulfonium salt formation, being attributable to the formation of the S^+CH_3 group. Concomitant downfield shifts of δ 0.08–0.09 for the two CH_2 groups flanking the sulfur atom were also observed, further supporting the S atom as the site of methylation. However, even after 24 h reaction time, S-methylation did not proceede to completion, only about 40% of 8a (X = I⁻) being formed (this

estimate is based upon NMR data). Longer reaction times resulted in the emergence of an extra singlet peak at δ 3.10, which was identical in value with the chemical shift of the S^+-CH_3 group of added S-methylmethionine iodide, indicating that decomposition of 8a (X = I⁻) to methionine, followed by S-methylation of the latter product, was occurring. When methyl sulfate in formic acid was used as the methylating agent, improved results were obtained, conversion to the methylsulfonium salt 8a being complete within 4 h, with no observable decomposition, even after 24 h at room temperature. Isolation of 8a from the reaction mixture was carried out by partitioning between water and diethyl ether, followed by lyophilization of the aqueous layer. The sulfonium salt 8b was obtained from 7b in a similar manner. Quantitative conversion of 11a,b to 12a,b, respectively, was also achieved by this procedure. No methylation of the adenine moiety was observed during the reaction conditions employed. The S^+-CH_3 signal in adenosyl derivative 12a was observed as two equiintense singlets at δ 2.90 and 2.87, while the 5'-CH_2 of the adenosyl moiety and the other CH_2 adjacent to the sulfonium pole had resonances centered at δ 3.80 and 3.36, respectively, which are 0.83- and 0.76-ppm downfield from the chemical shifts of these protons in the spectrum of the corresponding thioether 11a and provide further confirmation of methylation at the sulfur atom. The chemical shifts of the adenyl ring protons in both 12a and 11a were almost identical.

All of the above methosulfate salts were isolated as highly hygroscopic, white solids, which were converted to their corresponding perchlorate salts by anion-exchange chromatography.[31] Since sulfonium salts bearing three different ligands to the sulfur atom possess chiroptical properties,[34] the appearance of two close singlets for the S^+-CH_3 resonance in the NMR spectra of 12a–d indicates that these compounds each comprise 50:50 mixtures of their respective diastereomers.

Enzyme Inhibition Studies. The thioethers 7a,b and 11a,b and their methylsulfonium salts 8c,d and 12c,d were evaluated as inhibitors of rabbit lung INMT by radiochemical assay using S-adenosyl-L-[*methyl*-³H]methionine as methyl donor and tryptamine as substrate (see Experimental Section). The conversion of tryptamine to N-methyltryptamine was monitored by high-pressure liquid chromatography on two separatory systems (see Figures 1 and 2). None of the thioether compounds showed any significant inhibition of tryptamine N-methylation (see Table I). The homocysteinylsulfonium compound 8c exhibited only weak inhibition ($I_{50} \simeq 0.56$ mM), whereas 8d was completely inactive (see Table I). In contrast, the (5'-deoxy-5'-thioadenosyl)sulfonium compounds 12c,d showed good inhibitory activity. Compound 12c exhibited potent and competitive inhibition of INMT (see Figure 3 and Tables I and II) with a K_i of 12 μM, while the chain-contracted analogue, 12d, had a K_i of 44 μM (see Figure 4 and Tables I and II).

Discussion

With regard to the general line of approach in the design of these INMT inhibitors, the above results are very encouraging. The potent inhibition shown by 12c is paticularly interesting in view of the absence of the third large ligand to the sulfur atom in this compound, and it would seem to indicate that even greater inhibition may be achievable with sulfonium salts more closely related,

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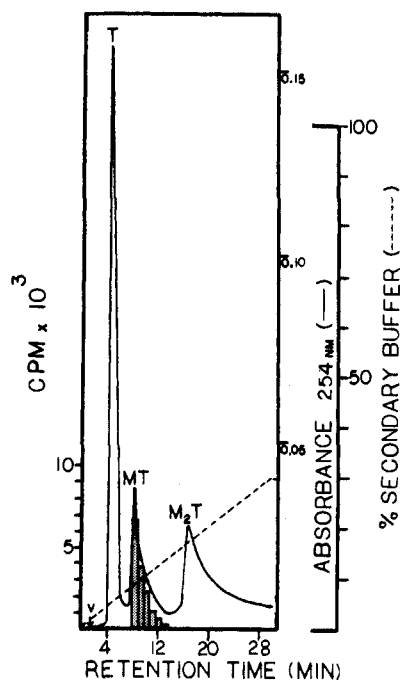
Figure 1. High-pressure liquid chromatogram obtained with a microparticulate siliceous cation-exchange column. Column: Partisil-10 SCX, 25 × 0.4 cm. Eluent: primary solvent, water; secondary solvent, 1.0 M acetate buffer (pH 4.5). The dotted line shows the gradient profile. Flow rate: 2.0 mL/min; inlet pressure: 1000 psig; temperature: ambient; fraction collection rate: 1 fraction/min; sample: 20 μL of lung supernatant extract with authentic markers; V = void volume. Note: 1-Methyltryptamine[41] exhibited a t_R value of 8.8 min on this analytical system.
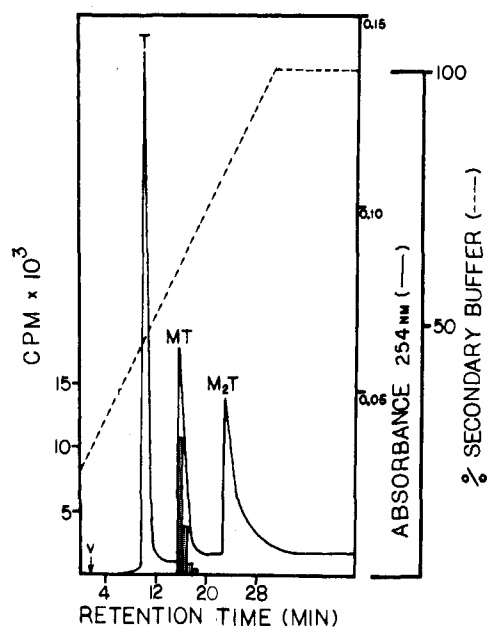

Figure 2. High-pressure liquid chromatogram obtained with a microparticulate siliceous reverse-phase column. Column: Partisil-10 ODS-1, 25 × 0.4 cm. Eluent: primary solvent, 0.05 M acetate buffer (pH 5.1); secondary solvent, 0.05 M acetate buffer (pH 4.6)/methanol (10:3, v/v). The dotted line shows the gradient profile. Flow rate: 2.0 mL/min; inlet pressure: 1000 psig; temperature: ambient; fraction collection rate: 1 fraction/min; sample: 20 μL of lung supernatant extract with authentic markers. V = void volume. Note: 1-Methyltryptamine[41] exhibited a t_R value of 22.8 min on this analytical system.

structurally, to the triliganded multisubstrate adduct **2**. Some interesting structure–activity considerations can be made from our present studies and those of others.[21,31] The lack of inhibitory activity of the thioether structures **7a,b**

Table I. Inhibition of INMT by Thioethers **7** and **8** and Methylsulfonium Salts **11** and **12**[a]

compd	I_{50},[b] μM
7a	$>1 \times 10^3$
7b	$>1 \times 10^3$
11a	$>1 \times 10^3$
11b	$>1 \times 10^3$
8c	560
8d	$>1 \times 10^3$
12c	38 ($I_{50}^{COMT} > 1 \times 10^4$ μM)[c]
12d	125 ($I_{50}^{COMT} > 1 \times 10^4$ μM)[c]

[a] Assays were carried out as described in the Experimental Section; 7.5 mg/mL of protein was used in each assay. Assays were incubated at 37 °C for 4 min. SAM concentration was 0.066 μM, tryptamine concentration was 1 mM, and inhibitor concentrations were 0.04–0.2 mM. Experiments were performed in duplicate, and the results had less than 10% standard deviation. [b] Determined from the three different concentrations of inhibitor and expressed as percent of drug-free control. [c] COMT inhibition was determined by using the method of Nikodejevic et al.[37] Purified porcine COMT equivalent to 1 mg/mL of protein was used in each assay. Assays were incubated at 37 °C for 4 min. 3,4-Dihydroxybenzoic acid concentration was 2 mM, and inhibitor concentrations were 0.5–20 mM.

Table II. Inhibition Constants for **12c** and **12d** toward INMT[a]

compd	inhibn constant, μM (K_i ± SD)[b,c]
12c	12.0 ± 0.82
12d	44.4 ± 2.8
SAH	2.0 ± 0.15
5-(methylthio)adenosine	700[d]

[a] Assays were carried out as described in the Experimental Section; 7.5 mg/mL of protein was used in each assay. Assays were incubated at 37 °C for 4 min. SAM concentrations used were 22.2, 35.7, 47.6, and 66.6 μM; tryptamine concentration was 1 mM. [b] Inhibition constants were determined by linear regression analysis. Each value is the result of at least two separate experiments. [c] Each inhibitor showed linear competitive kinetics. [d] Taken from ref 42.
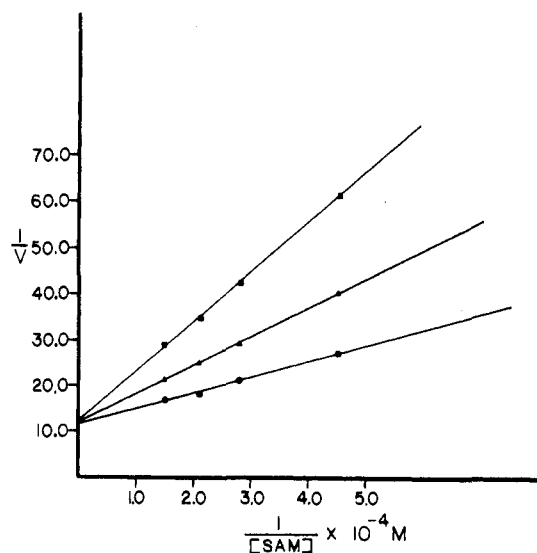

Figure 3. Lineweaver–Burk plot showing inhibition of rabbit lung INMT at 0 (O), 40 (△), and 100 μM (□) **12c**. Assay conditions are outlined in the Experimental Section. SAM concentration = 22.2–66.6 μM. Tryptamine concentration, 1 mM. Vel = nmol of product (mg of protein)$^{-1}$ min^{-1}.

and **11a,b** indicates that in the case of the INMT reaction, multisubstrate adducts bearing a positively charged sulfur atom are required for potent inhibition. Similar results
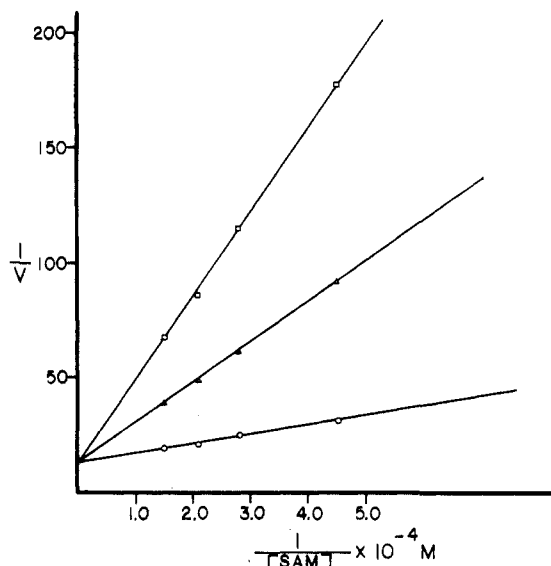
Figure 4. Lineweaver–Burk plot showing inhibition of rabbit lung INMT at 0 (●), 40 (▲), and 100 μM (■) **12d**. Assay conditions are outlined in the Experimental Section. SAM concentration = 22.2–66.6 μM. Tryptamine concentration, 1 mM. Vel = nmol of product (mg of protein)$^{-1}$ min^{-1}.

have been reported by Anderson et al.[31] for the inhibition of COMT by multisubstrate adducts, the thioether **13**

13

14a, R = CH(OH)N(CH₃)CH₂Ph
  b, R = H

15
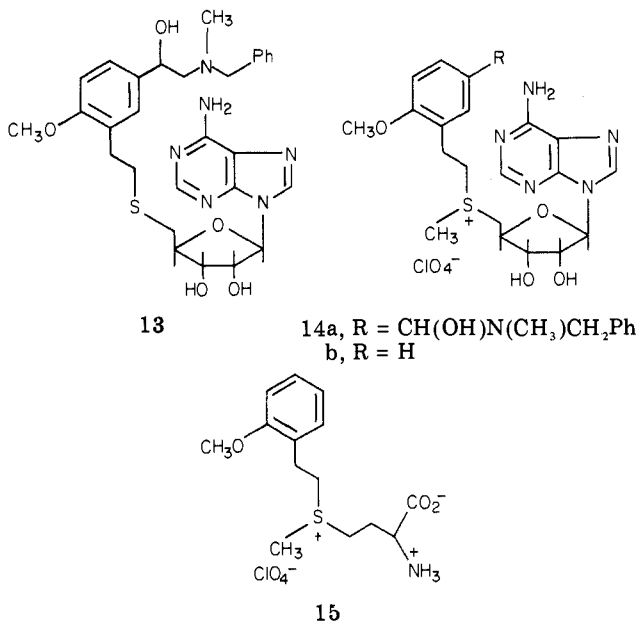
being ineffective as an inhibitor of this enzyme, whereas the corresponding methylsulfonium salt **14a** exhibited a K_i of 0.5 mM. These observations are consistent with mechanistic studies of model reactions,[35,36] as well as enzyme-catalyzed methylation,[26] which indicate that the positive charge on the sulfur atom is only partially removed in the transition state.

The poor inhibitory activity exhibited by the homocysteinylsulfonium compounds **8c,d** compared to the potent inhibition shown by **12c,d** suggests that the adenosyl moiety may be an important structural feature in the design of multisubstrate adduct inhibitors of INMT. This observation may not be restricted just to INMT inhibitors

but could be a requirement for the inhibition of other SAM-dependent methylases by transition-state analogues, since Anderson et al.[31] have reported that the inhibition of COMT by the (5'-deoxy-5'-thioadenosyl)sulfonium derivative **14b** is superior (K_i = 0.8 mM) to that shown by the corresponding homocysteinylsulfonium compound **15** (K_i = 5.8 mM). The weak inhibitory properties of the COMT inhibitors **14a,b** are worthy of note, in view of the potent inhibition of INMT shown by **12c**, and would seem to indicate that rather strict specificity for the structural features of the methyl acceptor moiety in multisubstrate adducts of this type may be required, since the ligand representing the catechol moiety in either **14a** or **14b** is not an exact structural equivalent of the catechol substrate. Clearly, more structure–activity studies on inhibitors of this type are required to determine the structural requirements for potent enzyme inhibition.

The inhibitory activity shown by the chain-contracted analogue **12d** may be explainable, of one assumes that in the transition state for the INMT reaction, a degree of compression occurs down the N–CH₃–S reaction coordinate, resulting in interatomic distances less than those for normal N–C and C–S bonds, effectively shortening the distance between the indole moiety and the sulfur atom in the transition-state structure depicted in **2**. This explanation is consistent with an observed compression of the O–CH₃–S grouping in the transition state for the COMT reaction,[27] the O–CH₃ and S–CH₃ distances calculated from kinetic isotope effect studies reflecting compressions of the order of at least 0.15 Å per bond, and possibly twice this amount, relative to the interatomic distances calculated for the O–C and S–C bonds in the model reaction transition state.

In order to determine the specificity of compounds **12c,d** for the INMT enzyme, these compounds were also evaluated as inhibitors of purified porcine COMT by the radiochemical assay of Nikodejevic et al.,[37] which utilizes 3,4-dihydroxybenzoic acid and S-adenosyl-L-[*methyl*-³H]methionine as substrates. Neither of the above compounds showed any significant inhibition of this SAM-dependent enzyme (see Table I).

In conclusion, these data from our preliminary studies show that potent and specific inhibition of INMT can be obtained with inhibitor molecules that have been designed on mechanism-based principles. One of these compounds, **12c**, is almost as effective an inhibitor of INMT as S-adenosyl-L-homocysteine (SAH) (Table II), the product inhibitor of most SAM-dependent methylases. This approach to inhibitor design may be useful in other SAM-dependent enzyme-catalyzed methyl-transfer reactions. We are actively pursuing synthetic routes to the target compound **2**, and work is in progress to evaluate the inhibitory properties of **12c** against other SAM-dependent methylases, in order to fully evaluate the specificity of this new class of methyltransferase inhibitor.

Experimental Section

Melting points were determined on a Reichert hot-stage microscope and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of Manchester. Analytical results obtained for all compounds were within ±0.4% of theoretical values unless otherwise stated. ¹H NMR spectra were recorded on a Perkin-Elmer R12 B (a), a Varian Associates EM-360 (b), or a Varian Associates SC-300 (c) spectrometer; tetramethylsilane was used as internal standard or as external standard where indicated. Infrared spectra were determined as Nujol mulls on a Perkin-Elmer 237 grating

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spectrophotometer. High-pressure liquid chromatographic (HPLC) analyses were carried out on an Altex 420 Gradient HPLC system using an Altex Model 153 analytical optical unit operating at 254 nm. Samples were introduced via a Rheodyne loop injector. 3-Indolebutyric acid, 3-indolepropionic acid, tryptamine hydrochloride, and *N*-methyltryptamine were purchased from Aldrich Chemical Co., *N,N*-dimethyltryptamine was purchased from Sigma Chemical Co., and 5'-deoxy-5'-chloroadenosine was synthesized by a previously reported procedure.[38] Indole ring N-methylated tryptamine was kindly supplied by Dr. William B. Jakoby, National Institutes of Health, Bethesda, MD.

4-(3-Indolyl)butan-1-ol (4a). To a rapidly stirred suspension of lithium aluminium hydride (10.0 g, 0.27 mol) in anhydrous diethyl ether (500 mL) was added a solution of 3-indolebutyric acid (23.0 g, 0.113 mol) in anhydrous diethyl ether (1 L) at such a rate that gentle reflux was maintained. During the course of the addition a white suspension was formed. The mixture was then stirred under reflux for a further 3 h and then at room temperature overnight. The excess lithium aluminium hydride was decomposed by the slow addition of water (50 mL), followed by the addition of a 1:3 mixture of sulfuric acid (95%)/water (150 mL). The ether layer was separated, and the aqueous layer was extracted with diethyl ether (3 × 250 mL). The combined ethereal fractions were dried, and evaporation of the solvent afforded **4a** (20.0 g, 93%), mp 37–38 °C, as a waxy solid. (This compound has previously been obtained as an oil, which, upon attempted distillation under reduced pressure, gave tetrahydrocarbazole.[39]): ^{1}H NMR (CDCl$_3$) δ 7.83 (1 H, br s, D$_2$O exchangeable), 7.73–7.48 (1 H, m, indolic C$_7$ H), 7.38–6.98 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.89 (1 H, s, indolic C$_2$ H), 3.65 (2 H, t, CH$_2$O, J = 6 Hz), 2.75 (2 H, t, =CCH$_2$, J = 6 Hz), 2.11 (1 H, s, OH, D$_2$O exchangeable), 1.93–1.43 (4 H, m, CCH$_2$CH$_2$C).

3-(3-Indolyl)propan-1-ol (4b). By the same procedure described for the synthesis of 4-(3-indolyl)butan-1-ol, 3-indolepropionic acid (3b; 25.0 g, 0.132 mol) gave **4b** (19.0 g, 82%) as a pale yellow viscous oil: ^{1}H NMR (CDCl$_3$) δ 8.10 (1 H, br s, D$_2$O exchangeable), 7.73–7.45 (1 H, m, indolic C$_7$ H), 7.35–6.95 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.82 (1 H, d, indolic C$_2$H, J = 2 Hz), 3.60 (2 H, t, CH$_2$O, J = 6 Hz), 2.77 (2 H, t, =CCH$_2$, J = 6 Hz), 2.15–1.65 (2 H, m, CH$_2$CH$_2$CH$_2$); picrate mp 102 °C (lit.[40] mp 100–101 °C).

4-(3-Indolyl)but-1-yl Bromide (5a). A solution of phosphorous tribromide (5.76 g, 0.021 mol) in anhydrous diethyl ether (370 mL) was added dropwise to a stirred solution of **4a** (10.0 g, 0.053 mol) in anhydrous diethyl ether (900 mL) cooled to 0 °C in an ice–salt bath. The ice bath was then removed, and stirring was continued overnight at room temperature. The ethereal solution was decanted from the resulting gummy deposit that had formed on the sides of the flask and was partitioned first with water (400 mL) and then with 10%, w/v, aqueous sodium bicarbonate solution. The etheral layer was dried and evaporated to dryness at room temperature to give an amber oil (6.8 g), which showed two components (R_f 0.70 and 0.30) by TLC on silica gel sheets using diethyl ether as developing solvent. The oil was dissolved in diethyl ether (10 mL) and applied to the top of a dry column of silica gel (60–200 mesh) (3 × 40 cm). Elution of the column with diethyl ether and evaporation to dryness of the combined fractions showing a single spot by TLC at R_f 0.70 gave an amber oil, which rapidly crystallized on scratching to give **5a** (3.2 g, 23.9%): mp 58 °C; ^{1}H NMR (CDCl$_3$) δ 7.80–7.35 (2 H, m, NH and indolic C$_7$ H, reducing to 1 H on addition of D$_2$O), 7.30–7.00 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.75 (1 H, s, indolic C$_2$H), 3.92 (2 H, t, CH$_2$Br, J = 6 Hz), 2.67 (2 H, t, =CCH$_2$, J = 6 Hz), 2.10–1.55 (4 H, m, CCH$_2$CH$_2$C). Anal. (C$_{12}$H$_{14}$NBr) C, H, N.

3-(3-Indolyl)prop-1-yl Bromide (5b). By the same procedure described for the synthesis of 4-(3-indolyl)but-1-yl bromide, **4b** (18.0 g, 0.102 mol) gave **5b** (5.5 g, 22.6%; lit. 33%[40]) as an amber, viscous oil: ^{1}H NMR (CDCl$_3$) δ 7.86–7.26 (2 H, m, NH and indolic C$_7$ H reducing to 1 H on addition of D$_2$O), 7.20–6.96 (3 H, m,

indolic C$_4$, C$_5$, and C$_6$ H), 6.80 (1 H, d, indolic C$_2$H, J = 2 Hz), 3.26 (2 H, t, CH$_2$Br, J = 6 Hz), 2.80 (2 H, t, =CCH$_2$, J = 6 Hz), 2.40–1.83 (2 H, m, CH$_2$CH$_2$CH$_2$). This compound decomposes on distillation in vacuo.

***S*-[4-(3-Indolyl)but-1-yl] Thioacetate (9a).** A mixture of **5a** (0.93 g, 3.7 mmol) and potassium thioacetate (0.84 g, 7.4 mmol) in a 1:1 mixture of acetone and absolute ethanol (16 mL) was heated under reflux for 1.5 h. The dark yellow mixture was cooled and filtered, and the filtrate was evaporated to dryness at 40 °C. Water (30 mL) was added to the resulting residue, and the mixture was extracted into diethyl ether (2 × 30 mL). The combined ether extracts were washed with water (2 × 30 mL), dried over magnesium sulfate, and decolorized with charcoal. removal of the solvent under reduced pressure gave a yellow oil, which rapidly crystallized on scratching to afford **9a** (0.71 g, 78.7%), mp 55–56 °C, which was purified for analysis by column chromatography over silica gel (80–200 mesh), eluting with diethyl ether: IR ν_{max} (Nujol) 3380 (NH), 1660 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.94 (1 H, br s, NH, D$_2$O exchangeable), 7.71–7.40 (1 H, m, indolic C$_7$ H), 7.31–6.95 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.81 (1 H, d, indolic C$_2$H, J = 2 Hz), 2.87 (2 H, t, CH$_2$S, J = 7 Hz), 2.72 (2 H, t, =CCH$_2$, J = 6 Hz), 2.25 (3 H, s, COCH$_3$), 1.98–1.50 (4 H, m, CCH$_2$CH$_2$C). Anal. (C$_{14}$H$_{17}$NOS) C, H, N.

***S*-[3-(3-Indolyl)prop-1-yl] Thioacetate (9b).** By the same procedure described for the synthesis of *S*-[4-(3-indolyl)but-1-yl]thioacetate, **5b** (2.5 g, 10.5 mmol) gave **9b** (2.2 g, 89.9%) as a pale yellow viscous oil, which was purified for analysis by column chromatography over silica gel (80–200 mesh), eluting with diethyl ether: IR ν_{max} (liquid film) 3380 (NH), 1670 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 8.00 (1 H, br s, NH, D$_2$O exchangeable), 7.70–7.40 (1 H, m, indolic C$_7$ H), 7.35–6.98 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.89 (1 H, d, indolic C$_2$ H, J = 2 Hz), 2.92 (2 H, t, CH$_2$S, J = 7 Hz), 2.80 (2 H, t, =CCH$_2$, J = 7 Hz), 2.28 (3 H, s, COCH$_3$), 2.25–1.78 (2 H, m, CH$_2$CH$_2$CH$_2$). Anal. (C$_{13}$H$_{15}$NOS) C, H, N.

4-(3-Indolyl)butane-1-thiol (9c). A solution of **9a** (1.9 g, 7.7 mmol) in anhydrous diethyl ether (70 mL) was added dropwise to a stirred suspension of lithium aluminium hydride (1.5 g) in anhydrous diethyl ether (100 mL). The mixture was stirred under reflux for 2 h and then for an additional 2 h at room temperature. The excess lithium aluminium hydride was decomposed by the slow addition of water (20 mL), followed by the addition of 25% aqueous hydrochloric acid (60 mL). The ether layer was separated, washed with water (2 × 100 mL), and dried over magnesium sulfate, and the solvent was removed under reduced pressure at ambient temperature to give **9c** (1.3 g, 82.4%) as a foul-smelling, viscous oil, which was unstable in air: IR ν_{max} (liquid film) 3390 (NH), 2560 (SH) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.96 (1 H, br s, NH, D$_2$O exchangeable), 7.80–7.55 (1 H, m, indolic C$_7$ H), 7.43–7.05 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.95 (1 H, d, indolic C$_2$ H, J = 2 Hz), 2.95–2.30 (4 H, m, =CCH$_2$S), 2.05–1.55 (4 H, m, CCH$_2$CH$_2$C), 1.31 (1 H, t, SH, J = 7 Hz, D$_2$O exchangeable). This compound decomposed on attempted purification by distillation at reduced pressure. Satisfactory C, H, N, and S analyses for this compound could not be obtained.

3-(3-Indolyl)propane-1-thiol (9d). By the same procedure described for the synthesis of 4-(3-indolyl)butane-1-thiol, **9b** (2.1 g, 9.0 mmol) gave **9d** (1.6 g, 93.1%) as a viscous yellow oil: IR ν_{max} (liquid film) 3400 (NH), 2565 (SH) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.85 (1 H, br s, NH, D$_2$O exchangeable), 7.78–7.50 (1 H, m, indolic C$_7$ H), 7.36–7.00 (3 H, m, indolic C$_4$, C$_5$, and C$_6$ H), 6.89 (1 H, d, indolic C$_2$ H, J = 2 Hz), 2.83 (2 H, t, =CCH$_2$, J = 7 Hz), 2.65–2.35 (2 H, m, CH$_2$S, collapses to t on D$_2$O exchange), 2.22–1.78 (2 H, m, CH$_2$CH$_2$CH$_2$), 1.26 (1 H, t, SH, J = 7 Hz, D$_2$O exchangeable). Satisfactory C, H, N, and S analyses for this compound could not be obtained.

***S*-[4-(3-Indolyl)but-1-yl]homocysteine (7a).** Homocysteine thiolactone hydrochloride (1.9 g, 12.0 mmol) and sodium hydroxide (1.5 g, 36 mmol) were dissolved in water (30 mL) and heated under reflux for 10 min, under a nitrogen atmosphere. Compound **5a** (1.6 g, 6.0 mmol) was then added in one portion, and refluxing was continued for an additional 45 min. Neutralization of the cooled solution with glacial acetic acid gave a white precipitate, which was filtered off and washed with water, acetone, and then diethyl ether to give **7a** (1.4 g, 76.2%): mp 200–201 °C (reprecipitated from 0.5 N sodium hydroxide solution with glacial acetic acid and washed with water); IR ν_{max} (Nujol) 3400 (NH), 1600

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(C=O) cm^{-1}; ^{1}H NMR (0.4 N NaOD; external Me$_4$Si standard) δ 7.65 (1 H, dd, indolic C$_7$ H, J = 7 Hz), 7.45 (1 H, dd, indolic C$_4$ H, J = 7 and 1 Hz), 7.22 (1 H, t, indolic C$_5$ or C$_6$ H, J = 7 Hz), 7.13 (1 H, t, indolic C$_6$ or C$_5$ H, J = 7 Hz), 7.08 (1 H, s, indolic C$_2$ H), 3.25 [1 H, t, CH(COOH)(NH$_2$), J = 6 Hz], 2.78 (2 H, t, =CCH$_2$, J = 6 Hz), 2.60-2.40 (4 H, m, CH$_2$SCH$_2$), 1.94-1.46 (6 H, m, CH_2CH$_2$CH$_2$S and SCH$_2$CH_2CH); ^{13}C NMR (0.4 N NaOD; external Me$_4$Si standard) 182.46 (s, COOH), 136.79 (s, indolic C-3a), 127.83 (s, indolic C-7a), 122.43 (d, indolic C-2), 121.99 (d, indolic C-5), 119.22 (d, indolic C-4 and C-6), 115.94 (s, indolic C-3), 111.92 (d, indolic C-7), 56.27 [d, CH(COOH)(NH$_2$)], 36.06 32.05, 29.50 (3 t, two superimposed signals), 28.91 and 24.83 (2 t, CH$_2$CH$_2$CH$_2$CH$_2$SCH$_2$CH$_2$ carbons) ppm. Anal. (C$_{16}$H$_{22}$O$_2$N$_2$S) C, H, N.

S-[3-(3-Indolyl)prop-1-yl]homocysteine (7b). By the same procedure described for the synthesis of S-[4-(3-indolyl)but-1-yl]homocysteine, **5b** (0.8 g, 3.36 mmol) gave **7b** (0.95 g, 96.8%): mp 195-196 °C (reprecipitated from 0.5 N sodium hydroxide solution with glacial acetic acid and washed with water); IR ν_{max} (Nujol) 3400 (NH), 1650 (C=O) cm^{-1}; ^{1}H NMR (0.4 N NaOD, external Me$_4$Si standard) δ 7.65 (1 H, dd, indolic C$_7$ H, J = 7 and 1 Hz), 7.45 (1 H, dd, indolic C$_4$ H, J = 7 and 1 Hz), 7.23 (1 H, t, indolic C$_5$ or C$_6$ H, J = 7 Hz), 7.14 (1 H, t, indolic C$_6$ or C$_5$ H, J = 7 Hz), 7.05 (1 H, s, indolic C$_2$ H), 3.25 [1 H, t, CH(COOH)(NH$_2$), J = 6 Hz], 2.78 (2 H, t, =CCH$_2$, J = 6 Hz), 2.63-2.43 (4 H, m, CH$_2$SCH$_2$), 2.00-1.63 (4 H, m, CH_2CH and CH$_2$CH_2CH$_2$S), ^{13}C NMR (0.5 N NaOD; external Me$_4$Si standard) 182.32 (s, COOH), 136.44 (s, indolic C-3a), 127.39 (s, indolic C-7a), 122.43 (d, indolic C-2), 121.85 (d, indolic C-5), 118.93 (d, indolic C-4 and C-6), 114.84 (s, indolic C-3), 111.78 (d, indolic C-7), 55.90 [d, CH(COOH)(NH$_2$)], 35.62, 31.32, 29.86, 28.40, and 24.02 (5 t, CH$_2$CH$_2$CH$_2$SCH$_2$CH$_2$ carbons) ppm. Anal. (C$_{15}$H$_{20}$O$_2$N$_2$S) C, H, N.

5'-Deoxy-5'-[[4-(3-indolyl)but-1-yl]thio]adenosine (11a). Method A. A mixture of **9c** (0.8 g, 3.9 mmol) and 5'-deoxy-5'-chloroadenosine (1.2 g, 3.9 mmol) in 2 N aqueous sodium hydroxide (9 mL) was heated at 80 °C under a nitrogen atmosphere for 1 h. Neutralization of the cooled solution with glacial acetic acid resulted in the liberation of a gum from which the aqueous supernatant was decanted. The gummy residue, after washing several times by decantation with water, slowly crystallized to a tan solid when left to stand at 4 °C overnight under water. The solid was collected by filtration and allowed to dry in air, affording **11a** (0.85 g, 47.9%): mp 84-88 °C (from absolute ethanol-diethyl ether); IR ν_{max} (Nujol) 3900 (NH), 1640 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 10.01 (1 H, NH, D$_2$O exchangeable), 8.24 (1 H, s, adenyl C$_8$ H), 8.22 (1 H, s, adenyl C$_2$ H), 7.55 (1 H, dd, indolic C$_7$ H, J = 7 and 1 Hz), 7.38 (1 H, dd, indolic C$_4$ H, J = 7 and 1 Hz), 7.18 (1 H, s, indolic C$_2$ H), 7.07 (1 H, t, indolic C$_5$ or C$_6$ H, J = 7 Hz), 6.98 (1 H, t, indolic C$_6$ or C$_5$ H, J = 7 Hz), 6.72 (2 H, br s, NH$_2$, D$_2$O exchangeable), 6.02 (1 H, d, ribosyl C$_1$ H, J = 5 Hz), 4.92 (1 H, dd, ribosyl C$_2$ H, J = 6 and 5 Hz), 4.44 (1 H, dd, ribosyl C$_3$ H, J = 6 and 5 Hz), 4.30-4.18 (1 H, m, ribosyl C$_4$ H), 3.04-2.90 (4 H, m, reducing to 2 H with D$_2$O, ribosyl C$_5$ H, due to nonequivalence of the H's in this grouping, and 2 OH), 2.70 (2 H, t, =CCH$_2$, J = 6 Hz), 2.60 (2 H, t, CH$_2$CH_2S, J = 6 Hz), 1.84-1.58 (4 H, m, CCH$_2$CH$_2$C); ^{13}C NMR (0.4 N NaOD, external Me$_4$Si standard) 155.83 (s, adenyl C-6), 152.47 (d, adenyl C-2), 149.34 (s, adenyl C-4), 139.71 (d, adenyl C-8), 136.13 (s, indolic C-3a), 127.02 (s, indolic C-7a), 121.98 (d, indolic C-2), 120.59 (d, indolic C-5), 119.06 (s, adenyl C-5), 118.12 and 117.89 (2 d, indolic C-4 and C-6), 114.25 (s, indolic C-3), 111.18 (d, indolic C-7), 87.55 (d, ribosyl C-1), 84.05 (d, ribosyl C-4), 72.74 (d, ribosyl C-2 and C-3), 34.15, 31.89, and 29.05 (3 t, two superimposed signals), 24.23 (t, ribosyl C-5 and C$_4$H$_8$S carbons) ppm. Anal. (C$_{22}$H$_{26}$O$_3$N$_6$S·1H$_2$O) C, H, N.

Method B. A solution of **9a** (0.63 g, 2.55 mmol) in dimethyl sulfoxide (10 mL) was deoxygenated with a stream of nitrogen for 1 h, after which time 5'-deoxy-5'-chloroadenosine (0.68 g, 2.4 mmol) was added, followed by 4 N aqueous sodium hydroxide (2.0 mL, 8.0 mmol). The resulting solution was stirred at ambient temperature for 17 h and then poured into water (80 mL) to form a gummy deposit. The reaction mixture was then worked up as described in method A to give a tan solid (0.54 g, 46.6%), which had identical spectral properties and melting point with **11a** obtained by method A.

5'-Deoxy-5'-[[3-(3-indolyl)prop-1-yl]thio]adenosine (11b). A mixture of **9d** (1.0 g, 4.9 mmol) and 5'-deoxy-5'-chloroadenosine (1.4 g, 4.9 mmol) in 2 N aqueous sodium hydroxide (13 mL) was heated at 80 °C under a nitrogen atmosphere for 1 h. The solution was then worked up as described for the preparation of 5'-deoxy-5'-[[4-(3-indolyl)but-1-yl]thio]adenosine to give **11b** (1.3 g, 60.2%): mp 83-85 °C; ^{1}H NMR (acetone-d_6) δ 10.10 (1 H, br s, NH, D$_2$O exchangeable), 8.26 (1 H, s, adenyl C$_8$ H), 8.24 (1 H, s, adenyl C$_8$ H), 8.24 (1 H, s, adenyl C$_2$ H), 7.58 (1 H, dd, indolic C$_7$ H, J = 7 and 1 Hz), 7.39 (1 H, dd, indolic C$_4$ H, J = 7 and 1 Hz), 7.18 (1 H, s, indolic C$_2$ H), 7.10 (1 H, t, indolic C$_5$ or C$_6$ H, J = 7 Hz), 7.01 (1 H, t, indolic C$_6$ or C$_5$ H, J = 7 Hz), 6.66 (2 H, br s, NH$_2$, D$_2$O exchangeable), 6.04 (1 H, d, ribosyl C$_1$ H, J = 5 Hz), 4.96 (1 H, dd, ribosyl C$_2$ H, J = 6 and 5 Hz), 4.46 (1 H, dd, ribosyl C$_3$ H, J = 6 and 5 Hz), 4.26-4.18 (1 H, m, ribosyl C$_4$ H), 3.06-2.90 (4 H, m, reducing to 2 H with D$_2$O, ribosyl C$_5$ H, due to nonequivalence of the H's in this grouping, and 2 OH), 2.82 (2 H, t, =CCH$_2$, J = 6 Hz), 2.65 (2 H, t, CH$_2$CH_2S, J = 6 Hz), 2.01-1.86 (2 H, m, CH$_2$CH_2CH$_2$); ^{13}C NMR (0.4 N NaOD; external Me$_4$Si standard) 155.83 (s, adenyl C-6), 152.47 (d, adenyl C-2), 149.34 (s, adenyl C-4), 139.71 (d, adenyl C-8), 136.13 (s, indolic C-3a), 127.02 (s, indolic C-7a), 122.13 (d, indolic C-2), 120.67 (d, indolic C-5), 119.06 (s, adenyl C-5), 118.04 (d, indolic C-4 and C-6), 113.67 (s, indolic C-3), 111.26 (d, indolic C-7), 87.48 (d, ribosyl C-1), 83.98 (d, ribosyl C-4), 72.59 (d, ribosyl C-2 and C-3), 34.15, 31.82, 30.07, and 23.72 (4 t, ribosyl C-5 and C$_3$H$_6$S carbons). Anal. (C$_{21}$H$_{24}$O$_3$N$_6$S·1H$_2$O) C, H, N.

S-[4-(3-Indolyl)but-1-yl]-S-methylhomocysteine Methosulfate (8a) and Perchlorate (8c). Dimethyl sulfate (0.5 mL, 5.3 mmol) was added to a solution of **7a** (0.5 g, 1.63 mmol) in formic acid (4 mL). The solution was stirred at ambient temperature for 15 h and then partitioned between diethyl ether (40 mL) and water (40 mL). The aqueous layer was separated, washed with diethyl ether (4 × 40 mL), and lyophilized to give **8a** (0.64 g, 90.7%) as a hygroscopic fluffy powder: mp 103-107 °C; ^{1}H NMR (D$_2$O; external Me$_4$Si standard) δ 7.52-6.76 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 3.95-3.63 [4 H, m, CH$_3$SO$_4^-$ singlet at 3.70 superimposed on CH(COOH)(NH$_2$)], 3.46-2.49 (6 H, m, =CCH$_2$ and CH$_2$S$^+$CH$_2$) overlapping with 2.55 (3 H, s, S$^+$CH$_3$), 2.37-2.00 (2 H, m, CH$_2$CH), 1.98-1.28 (4 H, m, CCH$_2$CH$_2$C). Anal. (C$_{18}$H$_{28}$O$_6$N$_2$S$_2$·2H$_2$O) C, H, N. A solution of sodium perchlorate (50 mL, 0.1 M) was passed down a column of Dowex IX8-200 anion exchange resin (1 × 18 cm), and the column was then washed with water (150 mL). A solution of **8a** (0.18 g, 0.42 mmol) in water (15 mL) was passed through the column, which was then eluted with water (60 mL). Lyophilization of the collected eluant gave **8c** (0.12 g, 67.8%) as a hygroscopic fluffy powder: mp 115-119 °C; ^{1}H NMR (D$_2$O; external Me$_4$Si standard) δ 7.54-6.79 (5 H, m, C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 3.71 [1 H, t, CH(COOH)(NH$_2$), J = 6 Hz], 3.44-2.48 (6 H, m, =CCH$_2$ and CH_2S$^+$CH$_2$) overlapping with 2.52 (3 H, s, S$^+$CH$_3$), 2.36-2.00 (2 H, m, CH_2CH), 1.98-1.25 (4 H, m, CCH$_2$CH$_2$C). Anal. (C$_{17}$H$_{25}$O$_6$N$_2$SCl) C, H. N.

S-[3-(3-Indolyl)prop-1-yl]-S-methylhomocysteine Methosulfate (8b) and Perchlorate (8d). Dimethyl sulfate (0.5 mL, 5.3 mmol) was added to a solution of **7b** (0.5 g, 1.7 mmol) in formic acid (4 mL). The solution was stirred at ambient temperature for 15 h and then worked up as described for the preparation of **8a** to give **8b** (0.66 g, 92.2%) as a hygroscopic, fluffy powder: mp 102-106 °C; ^{1}H NMR (D$_2$O; external Me$_4$Si standard) δ 7.61-6.93 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$ and C$_7$ H), 3.90-3.61 [4 H, m, CH$_3$SO$_4^-$ singlet at 3.70 superimposed on CH(COOH)(NH$_2$)], 3.33-2.48 (6 H, m, =CCH$_2$ and CH_2S$^+$CH$_2$) overlapping with 2.53 (3 H, s, S$^+$CH$_3$), 2.36-1.73 (4 H, m, CH$_2$CH_2CH$_2$ and CH_2CH). Anal. (C$_{17}$H$_{26}$O$_6$N$_2$S$_2$·H$_2$O) C, H, N. This compound (0.11 g, 0.26 mmol) was then converted to its perchlorate salt by the method described for the preparation of **8c** to give **8d** (0.095 g, 89.8%) as a hygroscopic fluffy powder: mp 105-109 °C; ^{1}H NMR (D$_2$O; external Me$_4$Si standard) δ 7.58-6.85 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 3.73 [1 H, t, CH(COOH)(NH$_2$), J = 6 Hz], 3.35-2.50 (6 H, m, =CCH$_2$ and CH$_2$S$^+$CH$_2$) overlapping with 2.53 (3 H, s, S$^+$CH$_3$), 2.37-1.75 (4 H, m, CH$_2$CH_2CH$_2$ and CH_2CH). Anal. (C$_{16}$H$_{23}$O$_6$N$_2$SCl) C, H, N.

(5'-Deoxyadenosyl)[4-(3-indolyl)but-1-yl]methylsulfonium Methosulfate (12a) and Perchlorate (12c). Dimethyl sulfate (0.5 mL, 5.3 mmol) was added to a solution of **11a** (0.5 g, 1.1 mmol) in 95-97% formic acid (4 mL). The solution was stirred at

ambient temperature for 15 h and then worked up as described for the preparation of **8a** to give **12a** (0.5 g, 78.3%) as a hygroscopic fluffy white powder: mp 147–152 °C; ^{1}H NMR (Me$_2$SO-d_6) δ 10.50 (1 H, br s, NH, D$_2$O exchangeable), 8.43 (1 H, s, adenyl C$_8$ H), 8.23 (1 H, s, adenyl C$_2$ H), 8.66–7.83 (2 H, br s, NH$_2$ superimposed on adenyl C$_8$ and C$_2$ H, D$_2$O exchangeable), 7.53–6.70 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 5.93 (1 H, d, ribosyl C$_1$ H, J = 5 Hz), 4.83–4.06 (5 H, m, ribosyl C$_2$, C$_3$, and C$_4$ H, C$_2$ and C$_3$ OH) which with D$_2$O reveals 4.83–4.46 (1 H, m, ribosyl C$_2$ H) and 4.40–4.10 (2 H, m, ribosyl C$_3$ and C$_4$ H), 3.96–3.64 (2 H, m, ribosyl C$_5$ H), 3.57–3.16 (5 H, m, CH$_3$SO$_4^-$ singlet at 3.36 superimposed on CH$_2$CH_2S$^+$), 2.90 and 2.87 (3 H, two overlapped singlets, two diastereoisomeric forms of S$^+$CH$_3$), 2.76–2.36 (2 H, m, =CCH$_2$), 2.03–1.50 (4 H, m, CCH$_2$CH$_2$C). Anal. (C$_{24}$H$_{32}$O$_7$N$_6$S$_2$·1H$_2$O) C, H, N. This compound (0.12 g, 0.207 mmol) was then converted to its perchlorate salt by the method described for the preparation of **8c** to give **12c** (0.09 g, 76.4%) as a hygroscopic fluffy powder: mp 156–161 °C; ^{1}H NMR (Me$_2$SO-d_6) δ 10.53 (1 H, br s, NH, D$_2$O exchangeable), 8.43 (1 H, s, adenyl C$_8$ H), 8.20 (1 H, s, adenyl C$_2$ H), 8.56–7.66 (2 H, br s, NH$_2$ superimposed on adenyl C$_8$ and C$_2$ H, D$_2$O exchangeable), 7.50–6.70 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 5.95 (1 H, d, ribosyl C$_1$ H, J = 5 Hz), 4.80–4.50 (1 H, m, ribosyl C$_2$ H), 4.46–4.01 (4 H, m, ribosyl C$_3$ and C$_4$ H, C$_2$ and C$_3$ OH, reducing to 2 H with D$_2$O), 3.98–3.68 (2 H, m, ribosyl C$_5$ H), 3.51–3.14 (2 H, m, CH$_2$CH_2S$^+$), 2.88 and 2.85 (3 H, two overlapped singlets, two diastereoisomeric-forms of S$^+$CH$_3$), 2.69–2.34 (2 H, m, =CCH$_2$), 1.96–1.50 (4 H, m, CCH$_2$CH$_2$C). Anal. (C$_{23}$H$_{29}$O$_7$N$_6$SCl·4H$_2$O) C, H, N.

(5′-Deoxyadenosyl)[3-(3-indolyl)prop-1-yl]methylsulfonium Methosulfate (12b) and Perchlorate (12d). By the same procedure as described for the synthesis of **12a**, 5′-deoxy-5′-[[3-(3-indolyl)prop-1-yl]thio]adenosine (**11b**; 0.5 g, 1.14 mmol) gave **12b** (0.52 g, 80.5%) as a hygroscopic fluffy white powder: mp 153–158 °C; ^{1}H NMR (Me$_2$SO-d_6) δ 10.56 (1 H, br s, NH, D$_2$O exchangeable), 8.90 (2 H, br s, NH$_2$, D$_2$O exchangeable), 8.60 (1 H, s, adenyl C$_8$ H), 8.36 (1 H, s, adenyl C$_2$ H), 7.56–6.65 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 5.93 (1 H, d, ribosyl C$_1$ H, J = 5 Hz), 5.56 (2 H, br s, ribosyl C$_2$ and C$_3$ OH, D$_2$O exchangeable), 4.73–4.40 (1 H, m, ribosyl C$_2$ H), 4.39–4.11 (2 H, m, ribosyl C$_3$ and C$_4$ H), 4.05–3.69 (2 H, m, ribosyl C$_5$ H), 3.60–3.14 (5 H, m, CH$_3$SO$_4^-$ singlet at 3.36 superimposed on CH$_2$CH_2S$^+$), 2.90 and 2.87 (3 H, two overlapped singlets, two diastereoisomeric forms of S$^+$CH$_3$), 2.95–2.53 (2 H, m, =CCH_2 superimposed on S$^+$CH$_3$), 2.36–1.76 (2 H, m, CH$_2$CH_2CH$_2$). Anal. (C$_{23}$H$_{30}$O$_7$N$_6$S$_2$) C, H, N. This compound (0.15 g, 0.26 mmol) was then converted to its perchlorate salt by the method described for the preparation of **8c** to give **12d** (0.11 g, 76.2%) as a hygroscopic fluffy powder: mp 143–148 °C; ^{1}H NMR (Me$_2$SO-d_6) δ 10.83 (1 H, br s, NH, D$_2$O exchangeable), 9.16 (2 H, br s, NH$_2$, D$_2$O exchangeable), 8.81 (1 H, s, adenyl C$_8$ H), 8.43 (1 H, s, adenyl C$_2$ H), 7.62–6.66 (5 H, m, indolic C$_2$, C$_4$, C$_5$, C$_6$, and C$_7$ H), 5.96 (1 H, d, ribosyl C$_1$ H, J = 5 Hz), 5.33 (2 H, br s, ribosyl C$_2$ and C$_3$ OH, D$_2$O exchangeable), 4.84–4.53 (1 H, m, ribosyl C$_2$ H), 4.46–3.83 (4 H, m, ribosyl C$_3$, C$_4$, and C$_5$ H), 3.64–3.18 (2 H, m, CH$_2$CH_2S$^+$), 2.93 (3 H, 2 s, S$^+$CH$_3$), 2.90–2.56 (2 H, m, =CCH$_2$), 2.33–1.76 (2 H, m, CH$_2$CH_2CH$_2$). Anal. (C$_{22}$H$_{27}$O$_7$N$_6$S$_2$) C, H, N.

Enzyme Inhibition Studies. Male albino rabbits (2.0–2.22 kg) were sacrificed by ear-vein injection of a mixture of sodium pentobarbitone solution (4 mL; 50 mg/mL) and heparin sodium solution (0.25 mL; 10000 μm/mL). Lungs were removed, inflated with room air, and immediately perfused with 1.0 N saline (200 mL). Perfused lungs were homogenized in three parts of 1.15%, w/v, KCl solution, and the homogenate was centrifuged at 25000g for 45 min. The supernatant was dialyzed against 0.001 M sodium phosphate buffer, pH 7.9 (3 × 3 L), for 36 h. The final preparation was stored at −16 °C prior to use.

INMT activity of the dialyzed lung supernatant was determined by using S-adenosyl-L-[*methyl*-^{3}H]methionine (50 mCi/mmol, ICN Radiochemicals) and tryptamine hydrochloride as substrates. The incubation mixture (125 μL) contained lung supernatant (62.5 μL), tryptamine hydrochloride (12.5 μL, 2 mg/mL in 0.2 M phosphate buffer, pH 7.9) and S-adenosyl-L-[*methyl*-^{3}H]-methionine (17.5 μL, 50 μCi/mL in 1 mM HCl). Incubations were

carried out at 37 °C. In the inhibition studies, reactions were initiated by the addition of tryptamine hydrochloride in buffer to incubation mixtures containing lung supernatant, inhibitor, and varying concentrations of S-adenosyl-L-[*methyl*-^{3}H]-methionine at 37 °C, as indicated (see Figures 3 and 4). After 4 min, incubations were terminated by the addition of equivolumes of 0.125 M borate buffer, pH 10. A blank incubation was performed containing no tryptamine hydrochloride. Quenched incubates were each extracted with ethyl acetate (500 μL), the organic phase was separated and washed with 0.125 M borate buffer, pH 10.0, and the solvent was evaporated under a stream of nitrogen. The residue was taken up in 1.0 M sodium acetate buffer, pH 4.5 (260 μL), containing the standard markers, tryptamine hydrochloride, N-methyltryptamine, and N,N-dimethyltryptamine (1 mg/mL of each) and an aliquot (20 μL) of this solution injected onto a Partisil-10 SCX column (25 × 0.4 cm) (see Figure 1 for operating conditions). A second aliquot (20 μL) of the mixture was injected onto a Partisil-10 ODS column (25 × 0.4 cm) (see Figure 2 for operating conditions). Radioactive effluents were monitored by collecting fractions of known volume directly into scintillation vials. Sufficient Aquasol-2 scintillation cocktail (60–70%, v/v) was added to form a clear solution. Samples were analyzed for radioactivity on a Packard Tri-Carb Model C2425 scintillation spectrometer. Radiochromatograms were constructed by plotting the radioactivity of the fractions against the retention time of the indolamine standards. Recovery of radioisotope from analytical columns was determined by adding an identical volume of the radioisotopic material that had been applied to the column directly to a blank tube of column effluent generated by the fraction collector. In general, recoveries were always near quantitative. In all the incubate extracts examined on these two analytical systems, 98.0–99.5% of the radioactivity in the sample migrated with the N-methyltryptamine standard peak, whereas less than 0.4% of the radioactivity migrated with the N,N-dimethyltryptamine standard peak (see Figures 1 and 2).

Recent studies by Lyon and Jakoby[41] have shown that an arylamine N-methyltransferase can be isolated from rabbit liver, which converts tryptamine into 1-methyltryptamine; i.e., methylation occurs at the indolic N atom. The two analytical systems described above will distinguish between 1-methyltryptamine and side-chain N-methyltryptamine (MT). The former compound has t_R's of 22.8 and 8.8 min on reverse-phase and cation-exchange HPLC, respectively, while the latter compound exhibits t_R's of 16.2 and 8.6 min, respectively, on these analytical systems (for details, see Figures 1 and 2).

The activity of purified porcine catechol O-methyltransferase (Sigma Chemical Co., 1 mg of protein/mL) was determined with 3,4-dihydroxybenzoic acid (2 mM) as substrate by the method of Nikodejevic et al.[37] I_{50} values for inhibitor molecules were calculated from incubations performed in the presence of varying concentrations of inhibitor (0.5–20 mM) (see Table I).

(41) E. S. Lyon and W. B. Jakoby, *J. Biol. Chem.*, **257**, 7531 (1982).
(42) R. Porta, C. Esposito, and G. Della Pietra in "Biochemical and Pharmacological Roles of Adenosylmethionine in the Central Nervous System", V. Zappia, E. Usdin, and F. Salvatore, Eds., Pergamon Press, New York, 1979, pp 105–117.