after lyophilization. HPLC analysis for steviol of the two THF extracts showed none to be detectable. With a detection limit of 0.05  $\mu$ g, as little as 0.03 and 0.13% degradation to steviol could have been detected for the sediment and supernatant fractions, respectively.

As has been shown above, the sulfopropyl ester moiety is quite stable to the biological conditions which readily degrade the glucosyl ester of stevioside. In addition, we have found this functionality to be inert to boiling alkali and to be quite unreactive toward hot dilute sulfuric acid.<sup>12</sup> Clearly, in vivo formation of steviol from 4 is quite unlikely and, therefore, we expect that any toxicity related to steviol formation would be eliminated by use of sulfopropyl ester 4 rather than the biologically unstable stevioside. In addition, it should be noted that 4 exhibits (p < 0.003) a cleaner taste [sweet (S)/bitter (B), other (O) = 92/8] than stevioside (S/B, O = 62/38), and which appears (p < 0.32) to be somewhat better than that of saccharin (S/B, O = 85/15). In summary, we have synthesized a stevioside analogue which has been demonstrated by sensory panel studies to have a potent, clean sweet taste and which is expected to have improved safety for usage in food systems. Thus, we are presently pursuing the development of 4 for usage as a sweetener for all applicable food and medicinal systems.

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Articles

## Synthesis and Evaluation of Some Stable Multisubstrate Adducts as Inhibitors of Catechol O-Methyltransferase

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A new series of methylase inhibitors has been designed in which the nucleophilic methyl acceptor is attached to the adenosine and/or homocysteine fragments of the methyl donor, S-adenosylmethionine, to form a "multisubstrate adduct". In the present case, catecholamine analogues attached through a phenethyl sulfide linkage to 5'-thioadenosine or homocysteine have been synthesized, together with the corresponding methylsulfonium salts. These compounds were assayed as inhibitors of catechol O-methyltransferase, and the adenosylsulfonium salts (4) were found to be inhibitors of the enzyme.

The methylation of several biological nucleophiles by S-adenosylmethionine (SAM) is catalyzed by methylases specific for each nucleophile.<sup>1</sup> Recently, a myriad of analogues of both SAM<sup>2</sup> and S-adenosylhomocysteine (SAH),<sup>3,4</sup> itself a potent product inhibitor of nearly all SAM-dependent methylases, have been reported. The 7-deaza analogue of SAH, S-tubercidinylhomocysteine (STH), has been shown to be a potent inhibitor of RNA methylation and biogenic amine methylation in both cell free systems<sup>3,4</sup> and several cell culture systems.<sup>5-7</sup> Whereas both SAH and STH have nearly identical  $K_i$  values against several purified methylases, STH is more effective than SAH in cell culture systems. This has been ascribed to the stability of STH to the various enzymatic reactions responsible for SAH metabolism in mammalian cells.<sup>8</sup> While these results are encouraging in terms of inhibition of methylation in vivo, the general lack of specificity of SAH analogues warrants the investigation of an alternative approach. An exciting new approach to the design of highly potent and specific enzyme in inhibitors is the use of "transition-state analogues",<sup>9</sup> whose design is based

\*Address correspondence to Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12181. upon the chemical mechanism of enzyme action.

Catechol O-methyltransferase, COMT (EC 2.1.1.6), is a well-studied enzyme which methylates a relatively simple monomeric substrate, in contrast to many methyltransferases which act on macromolecular substrates.<sup>10</sup> Based upon kinetic and stereochemical studies of the COMT reaction and related model compound reactions,

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 Table I.
 Calculated vs. Observed <sup>13</sup>C NMR Spectra for 1,2,4-Trisubstituted Benzenes



<sup>a</sup> Carbon without hydrogen.

we have concluded the following:<sup>11-15</sup> (a) COMT-catalyzed transmethylation involves a direct nucleophilic attack on the methyl group of SAM. (b) The transition state is early to midway on the reaction coordinate; i.e., the carbon-sulfur bond of the methylsulfonium is only partially broken in the transition state. (c) This nucleophilic attack must be via a classic  $S_N^2$  reaction in which any deviation from a colinear arrangement of nucleophile  $CH_3-S^+ <$  in the transition state results in a dramatic decrease in the reaction rate. These data are incorporated in a representation of the proposed transition state of COMT shown in 1.



 $Ado = 5' \cdot deoxyadenos \cdot 5' \cdot yl$ 

It is evident that the bonds being broken and formed in 1 cannot be reproduced exactly in a stable analogue. However, a compound such as 2 should serve as a potential "transition-state analogue" or, more accurately, a multisubstrate adduct<sup>16</sup> for COMT. Our initial efforts directed toward the synthesis of 2 initially focused on the synthesis of a series of compounds (3 and 4) which incorporate the

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homocysteinyl or adenosyl moiety of 2, as well as the sulfonium center and a succeedingly more complex aromatic system for the "catechol" portion of 2. The substitution of the free phenol group of 2 with the methoxyl group in 3 and 4 in these initial studies is based upon model studies which indicate that an appropriately positioned free hydroxyl group can greatly enhance the decompositon rate of a nearby sulfonium center.<sup>13</sup> In this paper we describe the synthesis of the appropriate thioether precursors and their conversion to the sulfonium compounds 3 and 4. The results of in vitro inhibition studies of COMT by these compounds are also presented.

**Chemistry.** For the synthesis of sulfonium compounds, **3** and **4**, our general method utilizes the thioether which contains the two largest substituents, since the reactivity of alkylating agents toward thioethers, e.g., SAH, decreases with increasing chain length.<sup>17</sup> This approach would suggest the conversion of **5** to **6** as the source of the



substituents Y,  $R_1$ , and  $R_2$  as in 3 and 4

"catechol" portion of the requisite thioether precursors of 3 and 4. The facile oxidation of many thiols as liquids prompted our use of the stable crystalline thiouronium salts 6c,d or aminothiol hydrochlorides 6e-g. Compounds  $5a-d^{18-20}$  and  $6c^{21}$  have been reported previously. It became clear in the early stages of this work that stable

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Table II. Physical Properties of Substituted Phenethyl Mercaptan Derivatives (6)

no.	prep method	salt isolated	yield, %	mp, °C	formula	anal.
	A	HBr	85	96-98	C <sub>a</sub> H <sub>10</sub> N <sub>2</sub> S·HBr	C, H, N, S
6d	Α	HBr	59	100-102	C.H.N.OS HBr	C.H.N.S
6e	В	HCl	90	148-149	C.H.NO.S HCl	C.H.N.S
6 <b>f</b>	В	HCl	quant	oil	- 19 - 25 2	-,, -, -,
6g	В	HCI	78	120-124	C <sub>12</sub> H <sub>12</sub> NOS·HCl	C, H, N, S
no.	method	yield, %	mp,	°C	formula	anal.
10-			100.10	4		
19a 10h	č	30	193-18			$\mathbf{U}, \mathbf{H}, \mathbf{N}$
190	Ŭ	52	197-20	lu dec	$C_{11}H_{15}NO_2S_2$	$H, N, S; C^{\circ}$
19c	С	56	197-19	99 dec	$C_{12}H_{17}NO_{2}S$	C, H, N
19d	С	50	192-19	)5 dec	C <sub>13</sub> H <sub>10</sub> NO <sub>3</sub> S	C, H, N
19e	С	guant	glass			
19f	С	97	glass			
3c	F	67	99-10	)3	C.,H.,CINO,S	C. H. N
3d	$\mathbf{\bar{F}}$	86	109-11	.0	C <sub>14</sub> H <sub>22</sub> CINO,S·0.5H <sub>2</sub> O	C, H, N

<sup>a</sup> C: calcd, 51.33; found, 50.45.

Table IV. Physical Properties of 5'-Thioadenosines (24) and Sulfonium Salts (4)

no.	method	yield, %	mp, °C	formula	anal.
24c	D	quant	83-86	C <sub>18</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub> S·0.5H <sub>2</sub> O	C, H, N
24d	D	77	104-105	$C_{19}H_{23}N_5O_4SH_2O$	C, H, N
24e	$\mathbf{E}$	43	145-147	C <sub>20</sub> H <sub>30</sub> N <sub>6</sub> O <sub>5</sub> S CH <sub>3</sub> OH	C, H, N
4c	F	83	95-100 <i>ª</i>	C <sub>10</sub> H <sub>24</sub> ClN <sub>4</sub> O <sub>7</sub> S	C, H, N
<b>4d</b>	F	78	95-100 <sup>a</sup>	C <sub>20</sub> H <sub>2</sub> CIN <sub>4</sub> O <sub>4</sub> S	C, H, N
4e	F	79	ь	$C_{30}H_{40}Cl_2N_6O_{13}S\cdot 2H_2O$	C, H, N

<sup>a</sup> Preliminary softening. <sup>b</sup> Decomposes slowly over a range of ca. 110-130 °C.

sulfonium salts derived from 5a and 5b could not be obtained (vide infra). Therefore, the synthesis of more stable sulfonium salts derived from 5c-g and 6c-g was pursued. The synthetic routes to 5d-g and 6d-g starting from omethoxyphenethyl alcohol (7)<sup>18</sup> are outlined in Scheme I.

The Friedel-Crafts acylation of 7 resulted in acylation of the alcohol and monoacylation of the aromatic ring, which would be expected to occur para to the methoxyl group. The aromatic position of the <sup>1</sup>H NMR spectrum of the product indicates formation of a 1,2,4-trisubstituted benzene but does not allow differentiation of 8 from the alternate isomer 16. This was accomplished using <sup>13</sup>C



NMR spectroscopy. Use of the known ortho, meta, and para shift values of the acetyl group<sup>22</sup> on the observed absorbances of the aromatic carbons of 7 shows a marked difference in the calculated spectra of 8 and 16. The spectrum of the product is consistent only with that calculated for 8 (Table I). Bromination of 8 with Br<sub>2</sub> afforded the  $\alpha$ -bromoacetophenone 9, which shows the appearance of a sharp two-proton singlet at  $\delta$  4.37 and the disappearance of the three-proton singlet at 2.35  $\delta$  of 8. Condensation of 9 with methylamine resulted in formation of a dark, gummy material from which none of the desired product could be isolated.<sup>23</sup> However, condensation of 9 with N-methylbenzylamine gave almost quantitative conversion to the aminophenone 10, characterized only by <sup>1</sup>H NMR. Basic hydrolysis of the ester of 10 afforded 11, which was isolated as the oxalate salt, followed by reaction with PBr<sub>3</sub> to give 12, isolated as the HCl salt. Sodium borohydride reduction of 11 and 12 gave 13 and 5e, respectively. In each case, reduction of the ketone was apparent by the appearance of a one-proton multiplet at  $\delta$ 4.68 for the methine proton. Catalytic hydrogenolysis of the HCl salt of 5e in EtOH resulted only in debenzylation to give the HCl salt of 5f directly. Catalytic hydrogenolysis of 13 in acetic acid again removed the benzyl group, and continued hydrogenolysis in the presence of perchloric acid effected removal of the benzylic hydroxyl group<sup>24</sup> and esterification of the primary alcohol to give 14. Basic hydrolyses of the ester 14 gave 15, which was converted to 5g by use of PBr<sub>3</sub>. Reaction of 5e-g with thiourea, followed by basic hydrolyses of the thiouronium intermediates, afforded 6e-g, respectively, which were isolated as their HCl salts. The <sup>1</sup>H NMR spectra for compounds 5–15 are in complete accord with the assigned structures (see Experimental Section).

The synthesis of the sulfonium salts 3, containing the homocysteinyl moiety, is shown in Scheme II. The preparation of 19c from phenethyl bromide, 5c, and Sbenzylhomocysteine using sodium-liquid ammonia has been reported.<sup>25</sup> More recently, a simple method for the synthesis of S-alkylhomocysteines by the reaction of homocysteine thiolactone 17 with primary alkyl halides in sodium methoxide solution has been reported.<sup>26</sup> Appli-

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Scheme I



cation of this procedure to 5a-f afforded 19a-f. It was possible to isolate the crude methyl esters 18a-d as syrups, which were characterized by <sup>1</sup>H NMR spectroscopy. A nitrogen atmosphere was necessary to minimize the formation of homocystine when using 5e,f, since disulfide formation is known to be catalyzed by amines.<sup>27</sup>

The reaction of 19a and 19b with methyl iodide gave a complex mixture of products as determined by <sup>1</sup>H NMR and TLC, one of which appeared to be methylmethionine. The similarity of 3a and 3b to protonated acetals, which undergo facile cleavage in protic media, might account for these results. Methylthiomethyl esters have been shown to yield the corresponding acid via a sulfonium intermediate by treatment with methyl iodide and water in acetone.<sup>28</sup> Whereas 19b was stable in the methylation solvent,

Scheme II



formic acid, 19a underwent rapid decomposition. However, 19a was stable in a 3:1 mixture of acetic acid-formic acid, and thus methylation of 19a could be carried out. The resulting sulfonium, 3a, was unstable in the mixed acid medium.

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Scheme III



Alkylation of 19c-f with mehyl iodide in formic acid was conveniently followed by <sup>1</sup>H NMR spectroscopy, which showed the appearance of a sharp singlet at  $\delta \sim 3.08$  as the reaction proceeded. The isolated, light-sensitive iodide salts of **3c**,d were converted to the stable perchlorate salts by use of an ion-exchange resin. In each case, elemental analysis confirmed the incorporation of a single methyl group. The appearance of the three-proton singlet at  $\delta$ 3.08, in conjunction with a downfield shift of the methylenes  $\alpha$  and  $\beta$  to the sulfur atom in **3c**-g with respect to **19c**,d, confirmed that the sulfur atom was the point of alkylation. The magnitude of these shifts is  $\delta \sim 1.0$  and 0.40, respectively, for the  $\alpha$  and  $\beta$  methylenes. This same effect was noted when methionine was converted to Smethylmethionine under identical conditions.

Our initial attempts toward the synthesis of the nucleoside thioethers requisite for the syntheses of 4c-g involved the use of 5'-deoxy-5'-S-(thioacetyl)-2',3'-iso-propylidinyl-N-6-formyladenosine (20).<sup>29</sup> This stable



5'-thiol precursor was treated with sodium methoxide in methanol, followed by the addition of 5c, to give 21 in 37%yield after purification by preparative TLC to effectively remove the symmetrical disulfide 22. It is of interest to note the relative ease of formation of 22, in contrast to the analogous reaction of 5c with homocysteine thiolactone described above. Hydrolysis of the isopropylidine blocking group in formic acid, followed by alkylation with methyl iodide, afforded 4c as indicated by <sup>1</sup>H NMR.

The work of Kikugawa and colleagues makes possible the synthesis of 5'-[alkyl(aryl)thio]-5'-deoxyadenosines in two steps from adenosine.<sup>30,31</sup> As shown in Scheme III, reaction of 5'-deoxy-5'-chloroadenosine (23) with the appropriate thiol (6e) or thiol precursor (6c,d) afforded the thioethers 24c-e as stable crystalline compounds. Alkylation in formic acid with methyl iodide gave the sulfonium iodide salts, which were converted to the perchlorate salts of 4c-e. That alkylation had occurred at sulfur was indicated by the appearance of a new threeproton singlet at  $\delta \sim 3.08$ , accompanied by the downfield

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Table V. Inhibition of Catechol O-Methyltransferase<sup>a</sup>

compd	$K_{i}$ , <sup>b</sup> mM		
3c	not determined		
3d	5.8		
4c	0.8		
4d	0.8		
4e	0.5		
5'-deoxy-5'(methylthio)- adenosine	>5.4 mM <sup>c</sup>		
5'-deoxy-5'-(dimethyl- sulfonio)adenosine	stimulates enzyme <sup>c</sup> 6.0		

<sup>a</sup> Assayed by the method of Nikodjevic et al.<sup>31</sup> with saturating (2 mM) 3,4-dihydroxybenzoic acid and variable S-adenosylmethionine. <sup>b</sup> All compounds were competitive inhibitors with respect to S-adenosylmethionine. <sup>c</sup> Data from ref 33.

shift of the adjacent methylene signals in 4 vs. 24. In addition, control experiments using fragments of 24c-e(e.g., *N*-methylbenzylamine) demonstrated that no methylation at nitrogen occurred under the acidic conditions employed. Compound 4c prepared by the two methods (Scheme II or III) was identical by <sup>1</sup>H NMR and TLC and proved to be stable at ambient temperature for at least 9 days in D<sub>2</sub>O when monitored by <sup>1</sup>H NMR.

Enzyme Studies. The first-generation adducts 3 and 4 were evaluated as inhibitors of COMT using a standard radiochemical assay.<sup>32</sup> These compounds were competitive inhibitors with  $K_i$  values of  $\sim 1 \text{ mM}$  (Table V), whereas the corresponding thioethers, 19 and 24, were ineffective. While these values are considerably higher than would be expected from a tight-binding multisubstrate adduct, the fact that sulfonium compounds lacking either the adenosine (3) or homocysteine (4) of adduct 2 are active as COMT inhibitors is encouraging. The loss of either of the moieties from the product inhibitor, SAH, results in the complete loss of inhibitory activity.<sup>33</sup> Attachment of the third large ligand to either 19 or 24 to produce the adduct 2 is under investigation. It should be reemphasized that the compounds synthesized in the present work are weaker inhibitors of COMT than many of those described in the introduction [e.g., STH (3)]. However, this work represents the synthetic basis for our current efforts in the study of potent and specific multisubstrate adduct inhibitors of several methylases and aminopropyltransferases.<sup>34</sup>

## **Experimental Section**

All melting points were determined in open capillary tubes on a Thomas-Hoover Mel-Temp apparatus and are uncorrected. <sup>1</sup>H NMR spectra were recorded on a Varian T-60 spectrometer in the indicated solvent with Me<sub>4</sub>Si or DSS as internal standard. <sup>13</sup>C NMR spectra were recorded in a Bruker 90-MHz spectrometer in CDCl<sub>3</sub>. Chemical-shift values are in  $\delta$  (parts per million) from the internal standard. Elemental analyses were performed by Baron Consulting Co., Orange, CT, and unless otherwise stated, are within ±0.40% of the theoretical values for the elements shown. Homogeneity of all analytical samples was established by TLC on EM-5775 silica gel plates or Eastman no. 6065 cellulose plates, in at least two solvent systems. Anion-exchange resin AG1-X8, 100-200 mesh (chloride form), was purchased from Bio-Rad.

3-(2'-Acetoxyethyl)-4-methoxyacetophenone (8). To a suspension to  $AlCl_3$  (26.6 g, 200 mmol) and acetyl chloride (17 mL, 240 mmol) in 1,2-dichloroethane (300 mL) was added 7 (Aldrich; 15.2 g, 100 mmol) slowly while maintaining a temperature of <5 °C. The mixture was stirred at ambient temperature for

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3 h. After the dark mixture was poured into ice (300 g), the organic layer was extracted with H<sub>2</sub>O (3 × 100 mL), dried (MgSO<sub>4</sub>), filtered, and evaporated to give a dark oil. Distillation in vacuo afforded pure 8 as a colorless oil: bp 167–169 °C (1.7 mm); yield 18.3 g (78% yield); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.00 (3 H, s, O<sub>2</sub>CCH<sub>3</sub>), 2.53 [3 H, s, C(=O)CH<sub>3</sub>], 3.13 (2 H, t, J = 7 Hz, C<sub>6</sub>H<sub>3</sub>CH<sub>2</sub>), 3.88 (3 H, s, OCH<sub>3</sub>), 4.27 (2 H, d, J = 7 Hz, CH<sub>2</sub>O), 6.87 (H, d, J = 8.4 Hz, 5-C<sub>6</sub>H), 7.70–7.97 (2 H, m, 2,6-C<sub>6</sub>H<sub>2</sub>). Anal. (C<sub>13</sub>H<sub>16</sub>O<sub>4</sub>) C, H.

3-(2'-Acetoxyethyl)-4-methoxy- $\omega$ -bromoacetophenone (9). To a solution of 8 (6.90 g, 29.3 mmol) in CHCl<sub>3</sub> (100 mL) was added Br<sub>2</sub> (1.5 mL, 29.3 mmol). After stirring for 30 min at ambient temperature, the light orange solution was evaporated in vacuo to give an oil. Trituration with petroleum ether (50 mL, 30-60 °C) afforded crude 9 (8.85 g) as an off-white amorphous solid. Crystallization from EtOH (40 mL) gave pure 9 (6.9 g, 78% yield) as white crystals: mp 57-59 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.00 (3 H, s, O<sub>2</sub>CCH<sub>3</sub>), 2.97 (2 H, t, J = 7 Hz, C<sub>6</sub>H<sub>3</sub>CH<sub>2</sub>), 3.88 (3 H, s, OCH<sub>3</sub>), 4.25 (2 H, t, J = 7 Hz, OCH<sub>2</sub>), 4.37 (2 H, s, CH<sub>2</sub>Br), 6.87 (1 H, d, J = 8.4 Hz, 5-C<sub>6</sub>H), 7.70-7.97 (2 H, m, 2,6-C<sub>6</sub>H<sub>2</sub>). Anal. (C<sub>13</sub>H<sub>15</sub>BrO<sub>4</sub>) C, H.

3-(2'-Hydroxyethyl)-4-methoxy-ω-(N-methyl-N-benzylamino)acetophenone Oxalate (11). To a stirred solution of 9 (9.45 g, 30 mmol) in 2-butanone (200 mL) was added Nmethylbenzylamine<sup>16</sup> (7.20 g, 60 mmol). After 1 h, the precipitate (N-methylbenzylamine hydrobromide) was removed by filtration, and the filtrate was evaporated in vacuo. The oily residue was dissolved in  $Et_2O$  (300 mL), which was then extracted with  $H_2O$  $(3 \times 100 \text{ mL})$ , dried (MgSO<sub>4</sub>), and evaporated in vacuo to give **10** as a slightly yellow oil (3.50 g, 99%): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.00 (3 H, s, CH<sub>3</sub>CO<sub>2</sub>), 2.35 (3 H, s, NCH<sub>3</sub>), 2.93 (2 H, t, J = 7 Hz, C<sub>6</sub>H<sub>3</sub>CH<sub>2</sub>), 3.65 and 3.75 (4 H, 2 s, CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> and N-CH<sub>2</sub>CO), 3.87  $(3 \text{ H}, \text{ s}, \text{CH}_3\text{O}), 4.25 (2 \text{ H}, \text{ t}, J = 7 \text{ Hz}, \text{CH}_2\text{CH}_2\text{O}), 6.83 (1 \text{ H}, \text{ d}, \text{ H})$ J = 8.4 Hz, 5-C<sub>6</sub>H), 7.30 (5 H, s, C<sub>6</sub>H<sub>5</sub>), 7.70–7.97 (2 H, m, 2,6- $C_6H_2$ ). This oil was refluxed for 1 h in a mixture of 1.6 g (40 mmol) of NaOH in 165 mL of MeOH/H2O (10:1). Concentration in vacuo to  $\sim 20$  mL gave a mixture, which was dissolved in CHCl<sub>3</sub> (100 mL) and extracted with  $H_2O$  (3 × 50 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered, and evaporated in vacuo to afford crude 11 (8.85 g, 94% overall yield) as a colorless oil. The oil was dissolved in EtOH (100 mL) containing oxalic acid dihydrate (4.0 g, 32 mmol) and refluxed for 1 h. After the mixture cooled to ambient temperature and Et<sub>2</sub>O was added, the precipitate was filtered. A further crystallization from EtOH-Et<sub>2</sub>O gave the pure white oxalate salt of 11 (9.00 g, 74% overall yield): mp 157-159 °C dec; <sup>1</sup>H NMR of free base 11 (CDCl<sub>3</sub>) δ 2.32 (3 H, s, CH<sub>3</sub>N), 2.87 (2 H, t, J = 7 Hz,  $C_6H_3CH_2$ ), 3.30 (1 H, br s, OH,  $D_2O$ exchangeable), 3.60-4.00 (8 H, 3 s-1 t, OCH<sub>3</sub>, C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>N, N-C- $H_2CO$ , and  $CH_2OH$ ), 6.78 (1 H, d, J = 8.0 Hz, 5-C<sub>6</sub>H), 7.22 (5 H, s, C<sub>6</sub>H<sub>5</sub>), 7.63–7.93 (2 H, m, 2,6-C<sub>6</sub>H<sub>2</sub>). Anal. (C<sub>19</sub>H<sub>23</sub>NO<sub>3</sub>·C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N.

 $3-(2'-Bromoethyl)-4-methoxy-\omega-(N-methyl-N-benzyl$ amino)acetophenone Oxalate (12). To 11 oxalate (4.55 g, 11.3 mmol) in a 100-mL flask was added PBr<sub>3</sub> (2.45 mL, 25.8 mmol). After the initial vigorous foaming had subsided, the mixture was heated on a steam bath for 1 h. The resulting viscous oil was treated in portions with ice,  $CHCl_3$  (~100 mL total), and sufficient 4 N NaOH to give an upper, aqueous layer at pH 10-11. The organic layer was separated. The combined organic layers were extracted with saturated NaCl solution  $(2 \times 100 \text{ mL})$ , dried  $(MgSO_4)$ , filtered, and evaporated to give crude 12 as an oil, which was used directly for the preparation of 14. For analysis, the oxalate salt was prepared. The oil (200 mg, 0.59 mmol) was dissolved in EtOH (10 mL) containing oxalic acid dihydrate (90 mg, 0.70 mmol) and refluxed for 20 min. Et<sub>2</sub>O was added to the cloud point. Cooling overnight at 5 °C gave a white powder, which was recrystallized from EtOH-Et<sub>2</sub>O. Filtration afforded the oxalate salt of 12 (209 mg, 81%): mp 140-142 °C dec; <sup>1</sup>H NMR of free base 12 (CDCl<sub>3</sub>) δ 2.33 (3 H, s, NCH<sub>3</sub>), 2.97-3.30 (2 H, m, CH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>), 3.38-3.73 (2 H, m, CH<sub>2</sub>Br), 3.63 and 3.68 [4 H, 2 s,  $CH_2C_6H_5$  and N-CH<sub>2</sub>C(=O)], 3.87 (3 H, s, OCH<sub>3</sub>), 6.82 (1 H, d, J = 8 Hz, 5-C\_6H), 7.30 (5 H, s, C\_6H\_5), 7.70-8.00 (2 H, m, 2, 6-C\_6H<sub>2</sub>). Anal. (C<sub>19</sub>H<sub>22</sub>BrNO<sub>2</sub>·C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) C, H, N; Br: calcd, 17.14; found,

3-(2'-Hydroxyethyl)-4-methoxy-N-methyl-N-benzylphenylethanolamine (13). To a solution of 11 oxalate (1.21 g, 3.0 mmol) in MeOH (50 mL) was added 2 N NaOH (4.0 mL) and NaBH<sub>4</sub> (220 mg, 5.8 mmol), and the mixture was stirred at ambient temperature for 3 h. The pH was adjusted to ~6 with 3 N H<sub>2</sub>SO<sub>4</sub>, and the mixture was concentrated in vacuo to ~10 mL. H<sub>2</sub>O (50 mL) was added and the pH was adjusted to ~10 with NaOH solution, followed by extraction with CHCl<sub>3</sub> (2 × 50 mL). The combined organic layers were extracted with H<sub>2</sub>O (3 × 50 mL), dried (MgSO<sub>4</sub>), filtered, and evaporated to give 13 as a colorless oil (790 mg, 84%). This material was sufficiently pure for subsequent reactions: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.27 (3 H, s, CH<sub>3</sub>N), 2.52 (2 H, m, CHOHCH<sub>2</sub>-N), 2.83 (2 H, t, J = 7 Hz, CH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>), 3.23 (2 H, br s, 2 OH, D<sub>2</sub>O exchangeable), 3.52 and 3.60 (2 H, 2 s, CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 3.70 (5 H, s, t, OCH<sub>3</sub> + CH<sub>2</sub>OH), 4.63 (1 H, m, CHOH), 6.70 (1 H, d, J = 8 Hz, 5-C<sub>6</sub>H), 7.00-7.33 (7 H, m, 6-C<sub>6</sub>H<sub>2</sub> + C<sub>6</sub>H<sub>5</sub>).

3-(2'-Hydroxyethyl)-4-methoxy-N-methylphenethylamine Oxalate (15). The crude free base 13 (790 mg, 2.52 mmol) was dissolved in HOAc (25 mL) containing 10% Pd/C, and the mixture was shaken for 4 h at ambient temperature under  $H_2$  (40 psi). HClO<sub>4</sub> (70%, 0.5 mL) was added, and hydrogenolyses continued for 18 h at ambient temperature (40 psi). The mixture was filtered through Celite, KOAc (600 mg) was added, and the mixture was stirred for 1 h. The solid KClO<sub>4</sub> was removed by filtration. The filtrate was evaporated in vacuo to give an oily residue, which was mixed with  $H_2O$  (20 mL), adjusted to pH 10 with NaOH, and extracted with  $Et_2O$  (2 × 50 mL). The combined Et<sub>2</sub>O extracts were washed with  $H_2O$  (2 × 50 mL), dried (MgSO<sub>4</sub>), and evaporated to give 390 mg of oily 14: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ 1.87 (1 H, br s, NH), 1.97 (3 H, s, CH<sub>3</sub>CO<sub>2</sub>), 2.42 (3 H, br s, NCH<sub>3</sub>), 2.73 (4 H, br s, CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>3</sub>), 2.90 (2 H, t, CH<sub>2</sub>C<sub>6</sub>H<sub>3</sub>), 3.73 (3 H, s, OCH<sub>3</sub>), 4.23 (2 H, t, CH<sub>2</sub>O<sub>2</sub>CCH<sub>3</sub>), 6.58-7.12 (3 H, m, C<sub>e</sub>H<sub>3</sub>). The oil was refluxed in MeOH (40 mL)/1 N NaOH (10 mL) for 4 h, cooled, concentrated in vacuo, and extracted with CHCl<sub>3</sub> (3  $\times$  30 mL). The combined CHCl<sub>3</sub> were extracted with saturated NaCl  $(2 \times 30 \text{ mL})$ , dried (MgSO<sub>4</sub>), and evaporated in vacuo to give a colorless oil (270 mg). This was dissolved in EtOH (2 mL), and oxalic acid (160 mg) in EtOH (10 mL) was added to give an immediate precipitate, which dissolved on heating. The crystals which formed on cooling were filtered and washed with Et<sub>2</sub>O to give 15 (320 mg, 36%), mp 158-160 °C. Anal. (C12H19NO2·C2- $H_2O_4$ ) C, H, N.

2-(2'-Bromoethyl)anisole (5d). To 7 (7.50 g, 49.3 mmol) was added, dropwise, PBr<sub>3</sub> (2.50 mL, 26 mmol) slowly without cooling. The solution was heated on a steam bath for 1 h and then cooled. The oil was poured into ice and extracted with Et<sub>2</sub>O (2 × 200 mL). The combined Et<sub>2</sub>O extracts were extracted with saturated Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2 × 50 mL), saturated NaHCO<sub>3</sub> (2 × 50 mL), and then  $H_2O$  (2 × 50 mL). Evaporation in vacuo gave an oil, which was distilled in vacuo to give 5d (7.09 g, 67%) as a colorless oil: bp 105–107 °C (2.5 mm) [lit.<sup>18</sup> bp 126–127 °C (0.9 mm)]; <sup>1</sup>H NMR (CDCl<sub>3</sub>) 2.93–3.30 (2 H, m, CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 3.37–3.67 (2 H, m, CH<sub>2</sub>Br), 3.75 (3 H, s, OCH<sub>3</sub>) 6.63–7.93 (4 H, m, C<sub>6</sub>H<sub>4</sub>). Anal. (C<sub>9</sub>H<sub>11</sub>BrO) C, H, Br.

3-(2'-Bromoethyl)-4-methoxy-N-methyl-N-benzylphenylethanolamine Hydrochloride (5e). The crude amino ketone 12 (3.85 g, 10.5 mmol) was dissolved in MeOH (120 mL), followed by addition of 1 N NaOH (2 mL) and NaBH<sub>4</sub> (860 mg, 22 mmol). After the mixture was stirred overnight,  $3 \text{ N H}_2 \text{SO}_4$ was added slowly to pH 5-6. The residue obtained by concentration in vacuo to  $\sim 10 \text{ mL}$  was partitioned between CHCl<sub>3</sub> (100 mL) and  $H_2O$  (50 mL). The upper phase was adjusted to pH 10 with 4 N NaOH. The organic layer was separated and combined with the CHCl<sub>3</sub>  $(2 \times 100 \text{ mL})$  extracts of the aqueous layer. The organic phases were then extracted with saturated NaCl solution  $(2 \times 100 \text{ mL})$ , dried (MgSO<sub>4</sub>), filtered, and evaporated in vacuo to give the free base of 5e (3.70 g) as a colorless oil. After the oil was dissolved immediately in EtOH (100 mL), 12 M HCl (1.0 mL) was added. Evaporation in vacuo gave an oil, which was coevaporated twice more with EtOH to give an oily solid. The residue was dissolved in hot EtOH (40 mL), and then Et<sub>2</sub>O (100 mL) was added. The white crystals which formed overnight at 5 °C were filtered and washed with  $Et_2O$  to give pure 5e (2.52 g, 54%), mp 154-155 °C. Anal. (C<sub>19</sub>H<sub>24</sub>BrNO<sub>2</sub>·HCl) C, H, N.

3-(2'-Bromoethyl)-4-methoxy-N-methylphenylethanolamine Hydrochloride (5f). To a solution of 5e (1.24 g, 3.0 mmol) in EtOH (100 mL) was added a slurry of Pd/C (10%, 100 mg) in EtOH (10 mL). This was subjected to hydrogenation at 40 psi for 3 h. The suspension was filtered through Celite and washed with EtOH (50 mL). Evaporation in vacuo gave an oil, which was dissolved in hot EtOH (10 mL). Et<sub>2</sub>O was added to the cloud point and the mixture cooled at 5 °C overnight. Filtration gave **5f** (717 mg, 75% yield), mp 124–125 °C dec. Anal. (C<sub>12</sub>H<sub>18</sub>Br-NO<sub>2</sub>·HCl) C, H, N.

3-(2'-Bromoethyl)-4-methoxyphenethylamine Hydrochloride (5g). This was prepared by the same procedures as for 12, from 15 oxalate (299 mg, 1 mmol) and PBr<sub>3</sub> (0.22 mL, 2.3 mmol). The crude free base was dissolved in EtOH (15 mL), and 12 M HCl (0.10 mL) was added. Evaporation gave an oil, which was coevaporated with EtOH ( $2 \times 10$  mL) to give a white solid. This was dissolved in hot EtOH (10 mL), and Et<sub>2</sub>O (5 mL) was added slowly. After cooling to 5 °C overnight, the crystals were filtered and washed with Et<sub>2</sub>O to give 5g (133 mg, 40%), mp 131-132 °C. Anal. (C<sub>12</sub>H<sub>18</sub>BrNO-HCl) H, N; C: calcd, 46.70; found, 47.17.

Phenethyl Mercaptan Derivatives (6). Method A. To a solution of thiourea (3.80 g, 50 mmol) in EtOH (65 mL) was added 5c (9.25 g, 50 mmol) and the mixture refluxed overnight. The solvent was removed in vacuo to give an oil. Trituration with  $Et_2O$  afforded a white solid, which was filtered and washed with  $Et_2O$  to give 6c (11.11 g, 85% yield). Crystallization from hot EtOH, followed by the addition of  $Et_2O$  to the cloud point, gave 6c (5.43 g, 40%), mp 96–98 °C (Table II).

Method B. To EtOH (20 mL) and  $H_2O$  (10 mL) was added 5e (1.24 g, 3 mmol) and thiourea (456 mg, 6.0 mmol). After the mixture was refluxed overnight, 2 N NaOH (6 mL) was added and refluxing continued for 15 min. The mixture was poured into  $H_2O$  (20 mL) and extracted with CHCl<sub>3</sub> (3 × 30 mL), dried (MgSO<sub>3</sub>), filtered, and evaporated in vacuo to give a clear oil. This oil was dissolved in EtOH (30 mL) and 12 N HCl (0.30 mL) was added. Evaporation gave an oily solid, which was triturated in Et<sub>2</sub>O to afford 6e (900 mg, 90%) as a white solid. Crystallization from hot EtOH-Et<sub>2</sub>O gave an analytical sample of 6e, mp 148-149 °C (Table II).

Homocysteines (19). Method C. To 30 mL of MeOH in which Na (266 mg, 11.6 mmol) was previously dissolved was added 17 (918 mg, 6.0 mmol). After the mixture was stirred for 15 min, 5d (1.075 g, 5.0 mmol) was added, and the reaction was stirred for 1.5 h. Evaporation gave an oily white solid, which was triturated with  $Et_2O$  (100 mL) and filtered. Evaporation of the filtrate

gave the methyl ester as an oil (900 mg). This oil was dissolved in MeOH (10 mL), 1 N NaOH (10 mL) was added, and the mixture was stirred for 1 h. Evaporation to 10 mL, followed by neutralization to pH 7 with HCl, gave a white precipitate. Filtration afforded 19d (670 mg, 50%), mp 192–195 °C dec (Table III).

5'-Thioadenosines (24). Method D. To a solution of 400 mg (10 mmol) of NaOH in 15 mL of  $H_2O$  was added 1.45 g (5 mmol) of the isothiouronium salt 6d, and the resulting mixture was heated at 80 °C for 1 h under N<sub>2</sub>, at which time 570 mg (2.0 mmol) of 23 was added, and heating continued under N<sub>2</sub> for an additional 1 h. The reaction mixture was then cooled, the solution was adjusted to pH 6 with glacial HOAc, and the aqueous supernatant was decanted. The residue was triturated with Et<sub>2</sub>O to give a white solid, which was then crystallized from EtOH-Et<sub>2</sub>O, followed by recrystallization from EtOH-H<sub>2</sub>O, to yield 610 mg (77%) of 24d. An analytical sample was obtained after two recrystallizations from CH<sub>3</sub>OH, mp 104-105 °C (Table IV).

from CH<sub>3</sub>OH, mp 104–105 °C (Table IV). **Method E.** To a solution (5 mL) of 2 N NaOH previously purged with N<sub>2</sub> was added 285 mg (1 mmol) of 23 and 368 mg (1 mmol) of 6e. After heating under N<sub>2</sub> at 70 °C for 4 h, the reaction mixture was cooled and extracted with EtOAc ( $6 \times 5$  mL), and the dried organic extract was concentrated in vacuo. The resulting residue was dissolved in MeOH, and the desired product, 24e, slowly precipitated from solution: yield 250 mg (43%) of a white solid. Recrystallization from CH<sub>3</sub>OH gave an analytical sample, mp 145–147 °C (Table IV).

Sulfonium Salts (3 and 4). Method F. The appropriate thioether (1 mmol) was dissolved in formic acid (2.5 mL) and stirred in the dark with MeI (0.30 mL, 5 mmol) until the reaction was judged to be complete by <sup>1</sup>H NMR (3–72 h). The mixture was poured in ice (10 g) and extracted with Et<sub>2</sub>O (3 × 10 mL). The aqueous layer was lyophilized to give the iodide salt of the sulfonium compound, which was dissolved in H<sub>2</sub>O and passed through an anion resin column in ClO<sub>4</sub> form. The aqueous eluent was lyophilized to give the sulfonium perchlorate as a white powder (Tables III and IV).

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## Synthesis and Evaluation of Some Stable Multisubstrate Adducts as Specific Inhibitors of Spermidine Synthase

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A new series of aminopropyltransferase inhibitors has been designed in which the nucleophilic aminopropyl acceptor is attached to the aminopropyl donor, S-adenosyl-1-(methylthio)-3-propylamine (decarboxylated S-adenosylmethionine), to form a "multisubstrate adduct". In the present case, S-adenosyl-1,8-diamino-3-thiooctane (2b) and the corresponding methylsulfonium salt (3b) have been synthesized. Several compounds of this type were assayed as inhibitors of spermidine synthase, and both 2b and 3b were found to be potent inhibitors of the enzyme. The thioether 2b is the most potent inhibitor of spermidine synthase described to date and is almost totally devoid of inhibitory activity against the closely related aminopropyltransferase, spermine synthase. This type of compound should have use as a specific inhibitor of spermidine biosynthesis in vivo.

The polyamines spermidine and spermine are synthesized by a pair of aminopropyltransferases (APT), spermidine synthase and spermine synthase.<sup>1</sup> In these reactions, nucleophilic attack by either putrescine or spermidine at an electrophilic methylene carbon of decarboxylated S-adenosylmethionine (dcSAM) leads to the formation of the polyamine products spermidine and spermine, respectively. Our studies on the mechanism of enzymecatalyzed alkyl-transfer reactions have indicated that the S-adenosylmethionine (SAM) dependent methylase, cat-

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