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Polyamine Biosynthesis in Rat Prostate. Substrate and Inhibitor Properties of 7-Deaza Analogues of Decarboxylated *S*-Adenosylmethionine and 5′-Methylthioadenosine

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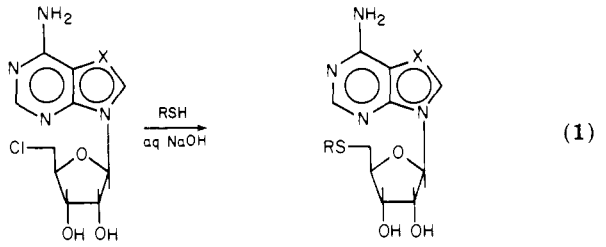
The 7-deaza analogue of 5′-methylthioadenosine, a nucleoside end product in polyamine biosynthesis, has been synthesized. This analogue has been shown to competitively inhibit the hydrolytic cleavage of the purine–ribose bond in methylthioadenosine with $K_i \simeq K_m$. In addition, the 7-deaza analogue of decarboxylated *S*-adenosylmethionine, a cofactor in the biosynthesis of both spermidine and spermine, has been synthesized. This analogue has been shown to act as a substrate in the reaction catalyzed by spermidine synthase, in which severe substrate inhibition by both the normal nucleoside substrate and the 7-deaza analogue is observed. These results are discussed in terms of possible end product regulation of polyamine biosynthesis and the possible substitution of the nucleoside antibiotic, tubercidin, for adenosine in reactions involving *S*-adenosylmethionine and its metabolites.

The role of *S*-adenosylmethionine (SAM, **4a**), and its various metabolites, in a myriad of cellular events has been extensively investigated in recent years[1] (Scheme I). As part of a program aimed at the regulation of SAM-dependent methylation, we have described the synthesis and in vitro activity of several analogues of *S*-adenosylhomocysteine (SAH, **5a**), a potent product inhibitor of most SAM-dependent methylases.[2-5] One of these compounds, *S*-tubercidinylhomocysteine (**5b**), the 7-deaza analogue of SAH, proved to be an extremely potent inhibitor of several SAM-dependent methylases.[4] Since our work[2-5] and others[1] have shown that only minor structural variations in the basic SAH structure can be made and still maintain inhibitory activity, we decided to study the 7-deaza derivatives of other SAM metabolites. Of particular interest is the possibility of regulating other biochemical reactions involving SAM metabolites, such as the biosynthesis of polyamines.[6] Previous work on synthetic inhibitors of these reactions has been directed at analogues of ornithine[7-12] and spermidine.[13,14] In addition, we have studied the action of *N*-(5′-phosphopyridoxyl)ornithine, an analogue of the reaction intermediate, on the enzyme ornithine decarboxylase.[15] However, no work has been done on analogues of the nucleoside substrates or products involved in polyamine biosynthesis.

Our rationale for synthesizing **5b**[4] and several other SAH analogues[2,3] was to incorporate a stable base–ribose bond into the molecule. Cleavage of the base–ribose bond is the major pathway of SAH metabolism in bacteria,[16] but recent studies in rat liver[17] and in stimulated rat lymphocytes[18] indicate that cleavage of the 5′-thioether to give homocysteine (**6**) and the nucleoside, **1**, is the major hydrolytic pathway in mammalian cells. However, in the case of polyamine biosynthesis, the nucleoside end product, 5′-methylthioadenosine (**9a**), is readily cleaved to adenine (**11a**) and 5-methylthioribose (**12**) by a phosphorylase (E.C. 2.4.2.) isolated from rat ventral prostate.[19] Thus an analogue of **9a** in which a stable base–ribose bond is incorporated should be resistant to the action of phosphorylases and should inhibit the hydrolysis of **9a** in vitro and, hopefully, in vivo. In this paper, we describe the synthesis of 5′-methylthiotubercidin (**9b**) and show that it is completely resistant to the action of the methylthioadenosine cleaving enzyme isolated from rat ventral prostate. As a result, **9b** competitively inhibits the enzyme-catalyzed cleavage of **9a**. Based on these findings, we have investigated the possibility that **9b** might be formed, and therefore accumulate, via the utilization of decarboxylated *S*-tubercidinylmethionine (**7b**) in the spermidine synthase (E.C. 2.5.1.16.) reaction.

Chemistry. 5′-Methylthioadenosine used in most previous biochemical studies has been obtained by acid hydrolysis of SAM.[19,20] The latter sulfonium compound is itself available only in limited amounts at considerable expense, so that a more economical route of synthesis for **9** was desired. Recent work by Kigugawa and colleagues[21,22] makes possible the synthesis of 5′-alkyl-(aryl-) thioadenosines in two steps from adenosine (eq 1). We have used this new procedure to prepare both [$^{14}CH_3S$]-**9a** and [$^{14}CH_3S$]-**9b**, in addition to the nonlabeled compounds.

Similarly, decarboxylated SAM (**7a**) used in biochemical studies has usually been obtained by enzymatic decarboxylation of SAM,[23,24] although **7a** has been synthesized by Jamieson[25] by coupling 5′-*O*-tosyl-2′,3′-isopropylideneadenosine and 3-(benzylthio)-1-propylamine in $Na–NH_3$. With a view toward synthesis of the 7-deaza analogue **7b**, we investigated alternate routes to decarboxylated SAM which did not use the intermediate 5′-*O*-tosyl derivative, since we had previously found 5′-*O*-tosyl-2′,3′-isopropylidenetubercidin to be quite unstable.[4] Considering the success achieved using the method of Kigugawa et al.[21,22] to prepare **9**, we sought unsuccessfully to prepare 3-phthalimidopropane-1-thiol[26] for use as an



13

9, R = CH_3

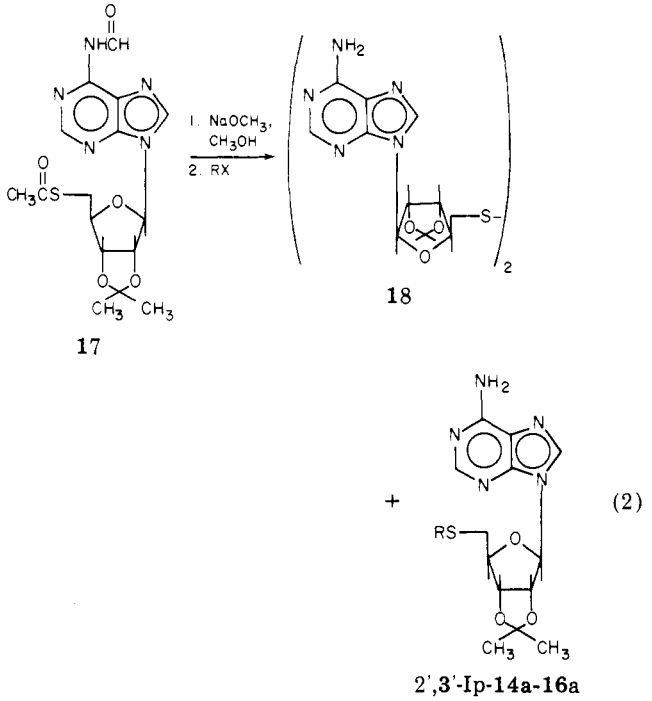
14, R = $-(CH_2)_3N$ (phthalimido)

15, R = $-(CH_2)_3NHCOCH_2Ph$

16, R = $-(CH_2)_3NH_2$

a, X = N; b, X = CH

intermediate in the synthesis of the decarboxylated SAM precursor **14a**. Similarly, attempts to generate the thiolate anion corresponding to *N*-carbobenzyloxy-3-bromo-1-propylamine[27] and then to couple it to 5'-chloro-5'-deoxyadenosine (**13a**) failed to give the desired sulfide **15a**. In other work from this laboratory,[28] we have used 5'-*S*-acetyl-5'-deoxy-5'-thio-2',3'-isopropylidene-N^6-formyladenosine (**17**)[29] as a stable precursor of the 5'-thiol, which can then be alkylated with an appropriate alkyl halide, according to eq 2. This appeared to be the method of



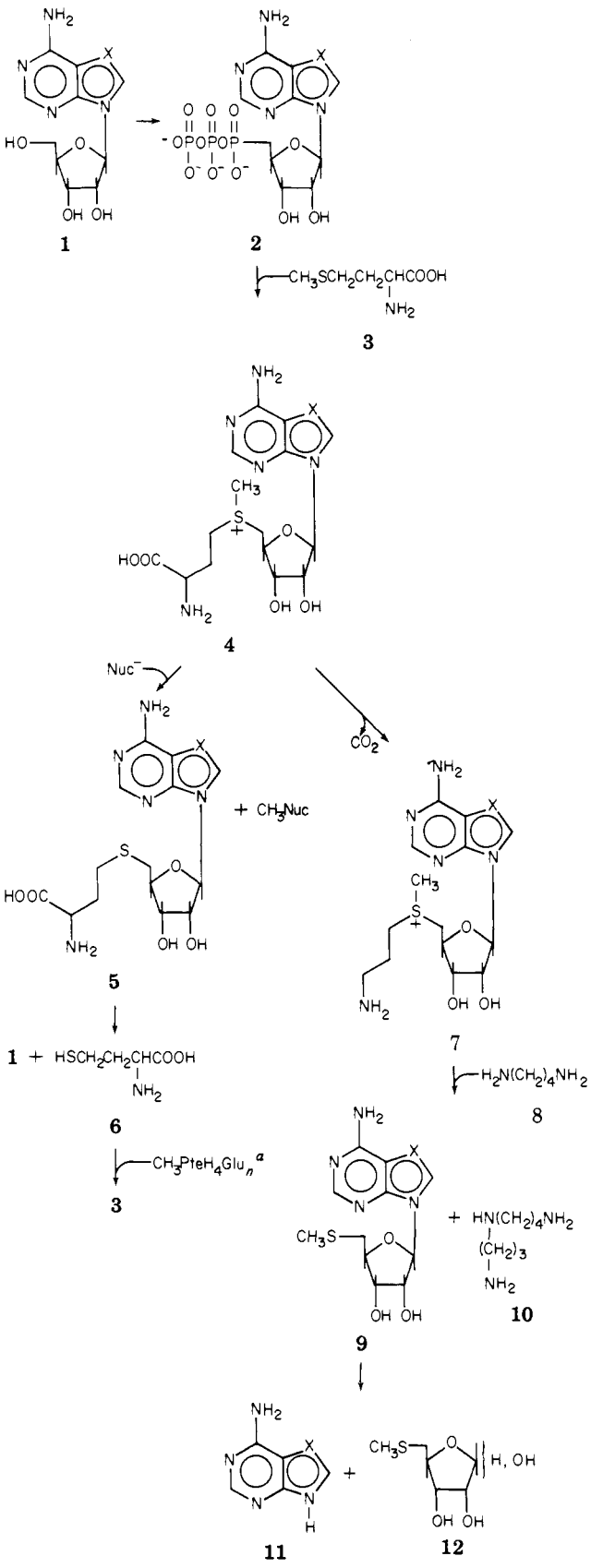
17

18

2',3'-Ip-**14a**-**16a**

choice for the synthesis of the 2',3'-isopropylidene (2',3'-Ip) derivative of **16a**, although purification of the desired product would require a tedious separation by preparative TLC to effectively remove the symmetrical disulfide **18**.[28] Attempts to deblock 2',3'-Ip-**15a** with HBr–HOAc led to complex reaction mixtures, from which the desired amino sulfide could not be isolated. However, removal of the phthalamide blocking group of 2',3'-Ip-**14a** with hydrazine to give 2',3'-Ip-**16a**, followed by methylation in acetic acid–formic acid, led to the desired compound **7a**. Despite the fact that 2',3'-Ip-**16a** could be prepared as described above, the low overall yield of **7a** from the parent nucleoside, adenosine, precluded the use of this route for synthesis of the 7-deaza analogue **7b** from the less readily available nucleoside, tubercidin. While this work was in

Scheme I



a, X = N; b, X = CH

[a] $CH_3PteH_4Glu_n$ = 5-methyl-5,6,7,8-tetrahydropteroyl poly-γ-L-glutamate[54]

progress, a report was published[30] in which the reaction shown in eq 1 was used to prepare SAH and related compounds. The reaction was carried out in liquid NH_3 and a thiolate anion generated in situ from homocystine. We have used this procedure to prepare **16** in good yield from **1** and 3-(benzylthio)-1-propylamine.[25] Methylation of **16** gave the desired sulfonium compounds, decarboxylated SAM, **7a**, and the 7-deaza analogue, **7b**.

Results and Discussion

To determine if **9b** is a substrate for methylthioadenosine phosphorylase, [$^{14}CH_3$]-**9b** was synthesized and used in place of [$^{14}CH_3$]-**9a** in the standard assay. No radioactive methylthioribose (<10 cpm) was formed from [$^{14}CH_3$]-**9b** (sp act. = 0.16 μCi μmol^{-1}) under conditions where appreciable amounts (2400 cpm) of this sugar was formed from [$^{14}CH_3$]-**9a** (sp act. = 0.12 μCi μmol^{-1}). Thus, in addition to their known resistance to nonenzymic acid hydrolysis,[31] tubercidin derivatives are resistant to enzyme-catalyzed hydrolysis of the heterocyclic base–ribose bond. To our knowledge, this is the first demonstration that a 7-deazaadenosine is not a substrate for the phosphorylase which cleaves the corresponding adenosine derivative, although this has generally been assumed to be the case based on chemical reactivity.[31] We then studied the effect of **9b** on the enzyme-catalyzed hydrolysis of **9a**. In Figure 1 are shown the results of such a study, and it is clear that **9b** acts as a competitive inhibitor of methylthioadenosine phosphorylase, with K_i = 0.6 mM (K_m^{9a} = 0.15 mM). Since **9b** does not act as an alternate substrate, and since K_i for **9b** is the same order of magnitude as K_m for **9a**, any **9b** that forms in the cell should effectively inhibit the hydrolysis of **9a**, providing of course that the cellular concentrations of **9a** and **9b** are similar. The latter proviso depends on the ability of exogenous tubercidin to be converted to **9b** via the pathway established[1] for the formation of SAM and its utilization in transmethylation reactions and polyamine biosynthesis (Scheme I).

As an initial step in establishing the ability of tubercidin to form **9b** via this pathway, we chose to study the reaction catalyzed by spermidine synthase. This enzyme catalyzes the transfer of a propylamino group from **7a** to putrescine (**8**). The products of the reaction are the polyamine spermidine (**10**) and **9a**. The literature describes several methods to follow this enzyme-catalyzed synthesis of spermidine.[24,32-36] The method described by Pegg and Williams-Ashman[24] utilizes small ion-exchange columns to effect the separation of putrescine and spermidine.[32] In our hands, the small Dowex 50 columns were unable to separate putrescine and spermidine under the assay work-up conditions previously described.[24] In fact, when radioactive putrescine (ca. 2 × 10^5 cpm) was applied to such a column, it was completely retained by the column, even after extensive (2 × 10 mL) washing with 1 N HCl; washing with 4 N HCl (2 × 10 mL) resulted in recovery of most of the applied radioactivity. Rather than use the tedious electrophoresis method,[33-35] or the coupled spectral assay,[36] we chose to use HPLC to separate putrescine and spermidine unambiguously. A detailed description of this method may be found in the Experimental Section.

Using synthetic **7a** as substrate, we established that the enzyme-catalyzed reaction was linearly dependent on time up to 60 min, after which the enzyme rapidly lost activity. When the concentration of **7a** was varied (0.01–1.0 mM), while maintaining a constant, high concentration (1 mM) of putrescine, a marked substrate inhibition was observed (Figure 2). When the concentration of the second substrate, putrescine (**8**), was varied (0.1–2.0 mM), while

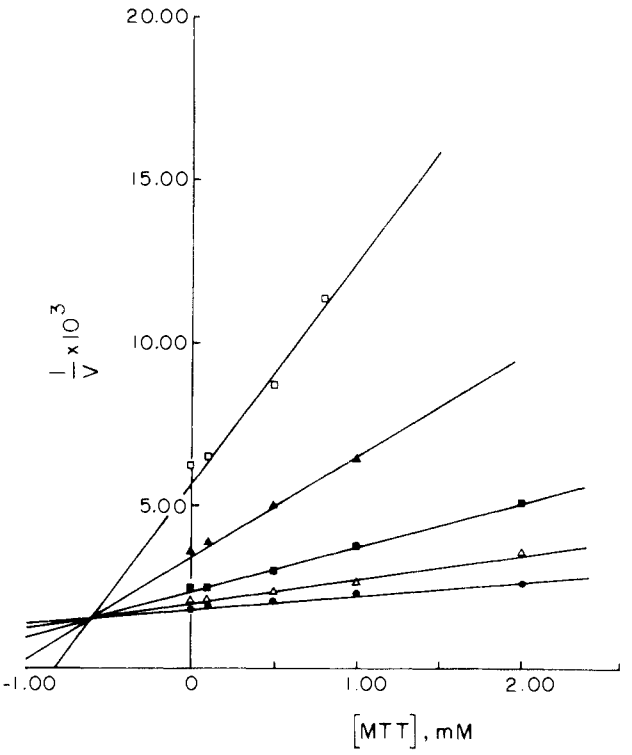


Figure 1. Dixon plot of inhibition of methylthioadenosine phosphorylase by **9b** (MTT) at concentrations of 0.05 (●), 0.10 (△), 0.25 (■), 0.50 (▲), and 1.0 mM (□) **9a**. [Protein] = 3.8 mg/ml; time of incubation = 30 min; temperature = 37 °C.

maintaining a constant, high concentration (0.1 mM) of **7a**, a more standard hyperbolic plot of v vs. [S] was observed, leading to a linear double-reciprocal replot (Figure 3). From the data of Figure 3, one can determine K_m = 0.24 mM for putrescine, in fair agreement with the previously reported value of 0.1 mM.[24] Analysis of the data of Figure 2 is more difficult, since one should not simply extrapolate the linear portion of the curve in order to obtain an "apparent K_m". As discussed by Cleland[37] and Segel,[38] substrate inhibition in a sequential, two-substrate reaction may be analyzed by a graphical procedure to fit eq 3. We were unable to obtain a unique fit to this

$$\frac{v}{V_{max}} = \frac{[A]}{K_a + [A] + [A^2]/K_i} \quad (3)$$

equation using all the data of Figure 2 for **7a**. However, by using the linear transform of Marmasse,[39] we could show that the data obtained at concentrations of **7a** greater than 0.025 mM fit eq 3 yielding values of K_a = 0.075 mM and K_i = 0.22 mM. The value of K_a may be compared with a value of K_m = 0.025 mM reported previously.[24] The more normal hyperbolic kinetics observed by Pegg and Williams-Ashman,[24] when **7a** was used as the variable substrate in the presence of 1 mM putrescine, did not include data with [**7a**] > 0.16 mM, where the pronounced substrate inhibition was observed in the present work. Although considerably more work must be done in the form of initial velocity and product inhibition studies on more highly purified preparations of spermidine synthase, these initial kinetic data demonstrate that this mammalian enzyme is much more complex than the bacterial counterpart.[36] In this regard, it should be noted that addition of 0.1 mM **9a** to the incubation mixtures at 0.025, 0.10, and 1.0 mM **7a** had little, if any, effect on the observed velocity of propylamine transfer, in agreement with a previously published statement.[40] Similarly, if 10 mM HEPES, pH 7.0, were used in place of 10 mM phosphate, pH 7.0, so as to

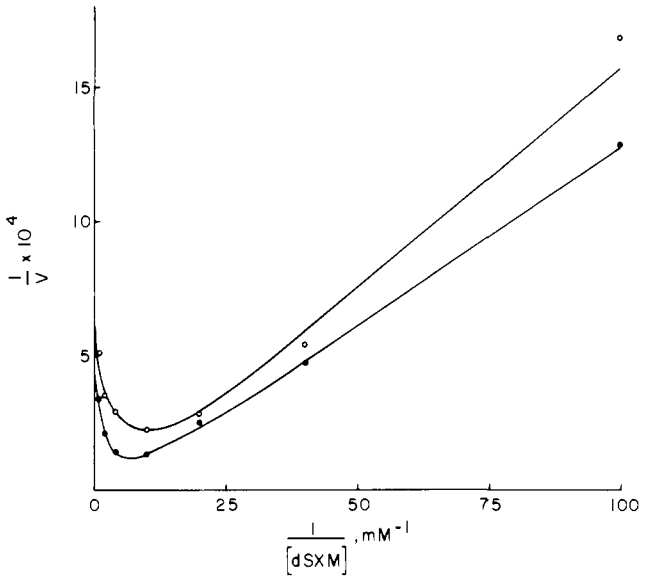


Figure 2. Lineweaver–Burk plot of data obtained using varied concentrations of **7a** (●) and **7b** (○) as substrates for spermidine synthase in the presence of 1 mM putrescine. [Protein] = 2.1 mg/ml; time of incubation = 60 min; temperature = 37 °C. dSXM = **7a** (dSAM) or **7b** (dSTM).

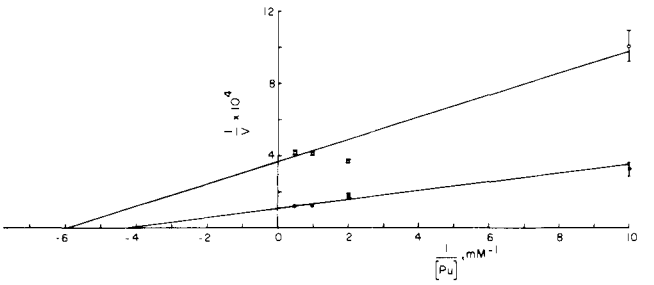


Figure 3. Lineweaver–Burk plot of data obtained using varied concentrations of putrescine as substrate for spermidine synthase in the presence of 0.1 mM **7a** (●) and **7b** (○). [Protein] = 3.1 mg/ml; time of incubation = 60 min; temperature = 37 °C. Pu = putrescine (**8**).

prevent phosphate-dependent hydrolysis of newly formed **9a**,[19] no appreciable difference in the rate of reaction was observed. Since conditions in all assays were such that conversion to products was routinely less than 2.5%, the absence of a HEPES effect is not surprising.

Using synthetic **7b** as substrate, we carried out studies similar to those described above for **7a**. Although the observed rate of spermidine synthesis was slightly lower using **7b**, the kinetic data were qualitatively similar (Figures 2 and 3). By an analysis similar to that described above, varying **7b** (0.025–1.0 mM) at constant, high putrescine concentration (1.0 mM) led to K_a = 0.021 mM and K_i = 0.48 mM. Similarly, varying putrescine (0.1–2.0 mM) at constant, high concentration (0.1 mM) of **7b** led to K_m = 0.17 mM.

Since the synthetic sulfonium compounds, **7**, are epimeric mixtures (± at the trivalent sulfur), it is possible that in the spermidine synthase reaction, as in other enzyme-catalyzed reactions at sp^3 carbon studied previously,[41-44] only one of the sulfonium enantiomers is active as a substrate, while the mirror image is not. It would be expected that if an enantiomer of a chiral substrate inhibited the appropriate enzyme, it would inhibit in a competitive manner, and this has been demonstrated in the case of thymidylate synthetase[45] and several SAM-dependent methylases.[46] If it is assumed that the inhibitory isomer of **7** acts as a competitive inhibitor of the active mirror image, the pronounced inhibition at high substrate concentration, shown in Figure 2, cannot be ascribed to the presence of an inhibitory enantiomer. At saturation, the active substrate would be capable of overcoming the effect of the inhibitory enantiomer, and thus a mixture of (±)-**7** would be expected to show normal hyperbolic kinetics, but with an increased K_m compared with the pure, active enantiomer. This type of kinetic behavior has been observed with the enantiomers of 5,10-methylenetetrahydrofolate, interacting with thymidylate synthetase.[47] Of all the possible modes of inhibition by the enantiomer of **7**, it is conceivable that one would fit the data of Figure 2 (e.g., parabolic, uncompetitive inhibition). However, the most plausible type of inhibition supported by precedent in the literature, i.e., competitive inhibition, does not explain the data of Figure 2.

It should be pointed out that the substrate inhibition of spermidine synthase by **7a** and **7b** (Figure 2) suggests another area for further study in the development of specific inhibitors of polyamine biosynthesis. Thus, the only reactions in which **7a** is known to act as a substrate are those involved in the biosynthesis of the polyamines, spermidine and spermine.[6] From the data presented in this paper, it appears that an analogue of **7a** which would not be susceptible to nucleophilic attack by putrescine would be an effective inhibitor of spermidine synthesis.

The data presented herein show that **7b** can participate as a substrate in the spermidine synthase reaction to yield spermidine (**10**) and **9b** which, unlike **9a**, is not further metabolized via hydrolytic cleavage of the purine–ribose bond or by deamination of the 6-amino group.[31] The cytotoxic nucleoside, tubercidin, can be converted to the corresponding 5'-triphosphate **2b**,[31] but it has not yet been determined whether this triphosphate can be converted to *S*-tubercidinylmethionine (**4b**) via an enzyme-catalyzed (E.C. 2.5.1.6.) "activation" of methionine (**3**). However, it has recently been shown that **4b** can participate in several transmethylation reactions in which *S*-adenosylmethionine (SAM, **4a**) functions as the natural methyl donor,[48] producing as one of the products, **5b**, a potent inhibitor of many SAM-dependent methylases.[4,49] The contrasting effects of **5a** and **5b** on DNA synthesis in PHA-stimulated rat lymphocytes[18] suggested to us that cleavage of the 5'-thioether bond was occurring in these long-term (50–60 h) cell cultures. However, recent data[50] obtained using purified SAH hydrolase (E.C. 3.3.1.1.) from rat liver suggest that **5b** is not a substrate for this enzyme-catalyzed hydrolysis. Formation of **7b** via enzyme-catalyzed (E.C. 4.1.1.50.) decarboxylation of **4b** has not been demonstrated in vitro or in vivo. In summary, of the enzyme-catalyzed reactions shown in Scheme I, only formation and decarboxylation of **4b** have yet to be studied. The data accumulated thus far suugest that 7-deaza analogues of adenosine derivatives can act as substrates in several enzyme-catalyzed reactions involved in the synthesis and utilization of SAM. However, since the products of transmethylation and polyamine synthesis, namely **5b** and **9b**, respectively, appear to be resistant to enzyme-catalyzed hydrolysis of the 5'-thioether and purine–ribose bonds, these inhibitors might accumulate in cells given tubercidin. Whether these metabolites are associated in any way with the effects elicited by tubercidin in whole cells has yet to be investigated.

Experimental Section[51]

All melting points are uncorrected. UV, IR, and NMR spectra were in accord with the assigned structure in all cases. Elemental microanalyses were carried out by Baron Consulting Company,

Orange, Conn. Analytical thin-layer chromatography (TLC) was carried out on precoated Eastman (6060) silica gel sheets and preparative thick-layer chromatography (PLC) on Brinkman (5766) 2-mm plates. 5′-Chloro-5′-deoxyadenosine (**13a**) and 5′-methylthio-5′-deoxyadenosine (**9a**) were prepared by literature procedures.[21,22] $^{14}CH_3SH$ (no. C-9999, Lot No. 784-204, sp act. = 12.5 μCi μmol^{-1}) and [1,4-^{14}C]putrescine dihydrochloride (no. NEC-150, sp act. = 13.6 μCi μmol^{-1}) were obtained from New England Nuclear. Tubercidin (**1b**) was a gift of Dr. George Whitfield, The Upjohn Co. Dowex AG 50W-X8, 100–200 mesh, H^+ form, was from Bio-Rad.

5′-Methylthio-5′-deoxytubercidin (9b). To a solution of thionyl chloride (655 mg, 5.5 mmol) in 2.66 mL of hexamethylphosphoric triamide (HMPT) was added 266 mg (1 mmol) of tubercidin. The reaction was carried out and worked up exactly as previously described for **9a**.[21] The purified product, 230 mg (81%), was homogeneous by TLC (R_f 0.60) on silica gel in 1-butanol–water (43:7). This material (112 mg, 0.4 mmol) was dissolved in a mixture of 1.0 mL of 2 N NaOH and 0.156 mL (0.4 mmol) of 20% (by weight) aqueous $NaSCH_3$ solution and then heated at 80 °C for 1 h. After cooling and acidification with acetic acid, the product was obtained as a brown precipitate which was collected on a filter and washed several times with cold water. The crude product was crystallized from water to give 30 mg (25%) of white needles: mp 172 °C; R_f 0.76 on silica gel in butanol–acetic acid–water (12:3:5); UV $\lambda_{max}^{0.1N\ H^+}$ 272 nm. Anal. ($C_{12}H_{16}N_4O_3S\cdot0.5H_2O$) H, N; C: calcd, 47.77; found, 47.21.

[$^{14}CH_3$]-5′-Methylthio-5′-deoxyadenosine ([$^{14}CH_3$]-9a). $^{14}CH_3SH$ (0.96 mg, 20 μmol, sp act. = 12.5 μCi μmol^{-1}) was converted to the sodium salt by quickly adding 0.2 mL of 2 N NaOH to the cooled (−70 °C) gas in a freshly opened ampule. The resulting solution was added to a flask containing 56 mg (0.2 mmol) of 5′-chloro-5′-deoxyadenosine (**13a**). The ampule was then rinsed with an additional 0.2 mL of 2 N NaOH which was added to the reaction flask, followed by 0.075 mL of a 20% (by weight) aqueous solution of nonradioactive $NaSCH_3$. The reaction solution was heated at 80 °C for 1 h, then cooled, acidified to pH 6 with acetic acid, and diluted to ca. 10 mL with H_2O. The resulting yellow solution was applied to a Dowex AG 50-X8 (H^+ form) column (1 × 7 cm) and the column washed with water (250 mL) to elute a small amount of UV-absorbing material (λ_{max} 260 nm). Then the eluent was changed to 1.0 N NH_4OH, and fractions of 2.8 mL were collected. The desired product was eluted in fractions 10–60; however, fractions 10–19 were shown to contain a radioactive impurity, and therefore fractions 20–60 were pooled and lyophilized to give a white, fluffy solid: $\lambda_{max}^{H_2O}$ 260 nm; R_f 0.76 on silica gel in butanol–acetic acid–water (12:3:5); yield, 18.5 μmol (9.2%); sp act. 0.24 μCi μmol^{-1}.

[$^{14}CH_3$]-5′-Methylthio-5′-deoxytubercidin ([$^{14}CH_3$]-9b). This compound was prepared in the same way as described above for radioactive **9a**, except that the desired product was obtained as a solid directly from the reaction solution, as described for nonradioactive **9b**. Additional product could be obtained from the reaction filtrate by PLC on cellulose in butanol–acetic acid–water (12:3:5). The material with R_f 0.7 was extracted from the cellulose with 0.1 N HCl and the aqueous extract lyophilized to give a white, fluffy solid: $\lambda_{max}^{0.1N\ H^+}$ 272 nm; total yield, 72 μmol (3.6%); sp act. 0.33 μCi μmol^{-1}.

***S*-Adenosyl-*S*-methyl-3-thioprop-1-ylamine (Decarboxylated *S*-Adenosylmethionine, 7a). A. From 17.** The nucleoside **17**[29] (2.0 g, 5 mmol) was dissolved in 200 mL of methanol containing 324 mg (6 mmol) of sodium methoxide. After stirring for 10 min at ambient temperature, the yellow solution was treated with 1.6 g (6 mmol) of 3-bromopropylphthalimide and allowed to stir overnight at ambient temperature. The solvent was then removed in vacuo and the oily residue partitioned between $CHCl_3$ and water. The dried organic extracts were concentrated in vacuo, and the desired product, 2′,3′-Ip-14a, separated from 18 by PLC on silica gel in EtOAc: yield 980 mg (38%) of a viscous straw-colored oil; R_f 0.44 on silica gel in EtOAc. The phthalimido compound, 2′,3′-Ip-14a (980 mg, 1.6 mmol), was dissolved in 200 mL of EtOH and 1.5 mL (25 mmol) of 85% hydrazine hydrate added. The resulting solution was heated at reflux for 18 h, after which the solvent was removed in vacuo to give an oily residue. This residue was dissolved in 20 mL of water and acetic acid was added to pH 4, and the turbid solution was clarified by filtration through a millipore filter. The filtrate was concentrated in vacuo, the pH adjusted to 11, and the solution extracted with $CHCl_3$. The dried organic extracts were evaporated in vacuo to give an oily residue: net wt 480 mg (82%); R_f 0.50 on silica gel in butanol–acetic acid–water (12:3:5). This oil was converted to the hydrogen sulfate salt of 2′,3′-Ip-**16a** as previously described:[25] net wt, 412 mg (21% overall from 17); mp 173–174 °C dec (lit.[25] 173–175 °C dec).

B. From 13a. To a three-necked round-bottom flask containing liquid NH_3 (25 mL) under a stream of N_2 was added 262 mg (1.4 mmol) of 3-(benzylthio)-1-propylamine,[25] followed by Na metal (ca. 150 mg) until the opaque blue color persisted for at least 15 min. Then **13a** (285 mg, 1 mmol) was added in one portion as the color changed from blue to a dull gold. After allowing the reaction mixture to stir at −70 °C for at least 6 h, the mixture was allowed to warm slowly as the NH_3 evaporated overnight. The dull gold residue was dissolved in water (25 mL), the solution neutralized to pH 6.0 with 12 N HCl, and filtered, and the filtrate was applied to two PLC plates. The plates were developed three times in butanol–acetic acid–water (12:3:5) and the band at R_f 0.6 was removed with CH_3OH. The gummy, orange residue obtained from the CH_3OH extracts was converted to the hydrogen sulfate salt of **16a** by treatment with 1 N H_2SO_4 as described above for the material obtained from **17**: yield, 55 mg (13% overall from **13a**); mp 173–174 °C dec.

The hydrogen sulfate of **16a**, prepared from either **13a** or **17**, could be methylated with methyl iodide in formic acid and acetic acid:[25] R_f 0.73 on cellulose in 5% aqueous Na_2HPO_4; $\lambda_{max}^{H_2O}$ 260 nm. After 10 min in 0.1 N NaOH, **7a** prepared in this manner had λ_{max} 268 nm, resulting from basic hydrolysis of the sulfonium nucleoside to adenine.[52]

***S*-Tuberdicinyl-*S*-methyl-3-thioprop-1-ylamine (Decarboxylated *S*-Tubercidinylmethionine, 7b).** This compound was prepared exactly as described above for the preparation of **7a** (method B), except that 5′-chloro-5′-deoxytubercidin (**13b**) was used as the starting material. In addition, the hydrogen sulfate salt of **16b** could not be isolated as a crystalline material in good yield, but the free amine was sufficiently pure for use in the methylation reaction. Methylation was accomplished with methyl iodide in formic acid and acetic acid[25] and **7b** isolated as a homogeneous salt: R_f 0.73 in 5% Na_2HPO_4; $\lambda_{max}^{H_2O}$ 270 nm.

Enzyme Isolation and Assay Procedures. The two enzymes of interest in this work, namely, methylthioadenosine phosphorylase (E.C. 2.4.2.)[19] and spermidine synthase (E. C. 2.5.1.16.),[24] were obtained by homogenization of ventral prostates from male Lewis/Mei rats under conditions previously described.[19,24] High-speed centrifugation (125 000g) of the crude homogenates yielded clear supernatants which were used as the sources for the two enzymes studied. Since it was found that ammonium sulfate fractionation and dialysis led to a substantial loss of enzyme activity, the undialyzed supernatant was used in order to assure maximum sensitivity in the determination of substrate or inhibitor activity of **7** and **9**. Reactions were found to be linear with time in the range employed in the kinetic studies and detailed in the figure legends. Methylthioadenosine phosphorylase was assayed by a procedure previously described.[19] and the radiolabeled substrates **9**, prepared as described above, were diluted with nonradioactive **9a** and **9b** in water and 5.0 mM HCl, respectively, to give 5.0 mM stock solutions with specific activities of 0.12 and 0.16 μCi μmol^{-1}, respectively. Spermidine synthase was assayed by the literature procedure,[24] except that determination of the amount of spermidine formed was accomplished as described below.

The quantitative determination of spermidine was based on the use of an amino acid analyzer to separate polyamines.[53] Thus, a modular HPLC system, consisting of a LKB Model 11300 Ultragrad gradient mixer and Milton-Roy minipump, was used with a linear salt gradient (1.5–5.0 M Na^+) over a 2-h period at 65 °C. The jacketed column (2 × 0.8 cm) was packed with a 9–10-μm, 10% crosslinked polystyrene B resin (ANC Laboratory, Walnut Creek, Calif.), the buffer was passed through this column, and fractions (2.7–2.8 mL each) were collected. The pump operated at 100 psig to give a flow rate of 1.08 mL min^{-1}. One reservoir contained distilled water and the other contained 1 N Na citrate, pH 6.18, and 4 M NaCl. A 0.2-mL aliquot of an assay supernatant[24] was injected into the pump line. After completing

a run, the column was equilibrated with 1.5 M NaCl for 15 min before injecting another aliquot. A 2.0-mL aliquot of each fraction was then added to 6 mL of Aquasol scintillation cocktail (New England Nuclear) to give a 25% gel, which was then counted in a Packard Tri-Carb scintillation spectrometer. Each assay and control were analyzed by plotting radioactivity vs. tube number, so that the amount of spermidine formed could be determined accurately by summing the total radioactivity in the spermidine peak (2.48 M Na^+). It was found that, in addition to the main peak of radioactive putrescine found at 1.88 M Na^+, a small amount (ca. 0.25%) of impurity eluted in the spermidine region. The radioactivity due to this impurity was routinely substracted from the total radioactivity detected in order to determine the net amount of radioactivity due to the formation and elution of spermidine. The recovery of radioactive putrescine from the column was found to be greater than 98%. By use of a standard nonradioactive mixture of the polyamines, putrescine, spermidine, and spermine, and detecting each by reaction with ninhydrin, it was possible to establish the identity of the radioactive peaks at 1.88 M Na^+ and 2.48 M Na^+ as putrescine and spermidine, respectively.

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