The structures of all compounds were confirmed by their IR, UV, and NMR spectra, the latter being determined as a solution in Me_2SO-d_6. The IR spectra were obtained with a Perkin-Elmer 237 spectrophotometer and the UV spectra with a Perkin-Elmer 137 spectrophotometer. The ^1H NMR spectra were obtained with a Varian Associates spectrometer Model A-60A and the ^{13}C NMR spectra were recorded on a JEOL PS100 spectrometer.

6-*tert*-Butylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide (8). 5-Amino-1,3,4-thiadiazole-2-sulfonamide (195.7 g, 1.087 mol) and 1-bromo-3,3-dimethyl-2-butanone (207.3 g, 1.087 mol) were heated under reflux in EtOH (1.5 L) for 60 h. After the mixture was cooled to 10 °C, **8** crystallized out from the reaction mixture as a hydrobromide salt and was collected by filtration and dried in vacuo at 50 °C: yield 290 g (78%); mp 278–280 °C.

The HBr salt was slurried in water (2.0 L) at room temperature and NH_4OH was added until solution was obtained. HCl (5 N) was added until the pH was 7.5, whereupon the precipitated solid was collected, recrystallized from EtOH, and dried in vacuo at 50 °C: yield 211.9 g (75%); mp 254–256 °C. Anal. ($C_8H_{12}N_4O_2S_2$) C, H, N. Pertinent spectral data for **8** are as follows: UV (MeOH) λ_{max} 262 nm; IR (Nujol) 3335, 3158, 1175, 1030, 920, 756, 709, 635 cm^{-1}; ^1H NMR 1.2 (9 H, s), 7.9 (1 H, s), 8.5 (2 H, m) ppm. With the exceptions of **5**, **21**, and **20**, all the compounds listed in Table I were made by this route using either of the aminoazoles **1** and an appropriate bromo ketone.

Imidazo[2,1-*b*]thiazole-6-sulfonamide (21). 2-Amino-4-sulfamoylthiazole hydrobromide (2.6 g, 0.01 mol) and diethyl bromoacetal (2.0 g, 0.01 mol) were heated under reflux for 12 h in EtOH (40 mL). Upon cooling, the crystalline solid was filtered, washed, and recrystallized from H_2O to give **21** as the HBr salt: yield 1.0 g (36%); mp >305 °C dec. Anal. ($C_5H_5N_3O_2S_2$·HBr) C, H, N.

Compound **5** was prepared in a similar manner using the appropriate thiadiazole and diethyl bromoacetal.

5-Phenylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide (20). 5-Amino-1,3,4-thiadiazole-2-sulfonamide (5.4 g, 0.03 mol) and redistilled α-bromophenylacetaldehyde (6.2 g, 0.03 mol) were heated under reflux for 12 h in EtOH (180 mL). Upon cooling, the crystalline solid was filtered, washed, and recrystallized from EtOH–H_2O to give **20**: yield 2.5 g (28%); mp 230–232 °C. Anal. ($C_{10}H_8N_4O_2S_2$) C, H, N.

1-Amino-2-mercapto-4-*tert*-butylimidazole (4). 6-*tert*-Butylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide (**8**; 13.4 g, 0.052 mol) was slurried in 5 N NaOH (100 mL), and the mixture was heated to 95 °C for 6 h. The solution was cooled and 5 N HCl was added until pH 7.0 was achieved. The resultant precipitate was collected, washed, dried, and recrystallized from EtOAc to give **4** as the HCl salt: yield 8.0 g (91%); mp 196 °C.

Acknowledgment. The authors thank M. J. Pitcher, R. F. Gammon, D. J. Tims, E. Hawkeswood, B. Petch, and S. Shevde for their able technical assistance. We also thank Dr. M. J. Sewell and his staff for analytical and spectral data.

Inhibitors of Polyamine Biosynthesis. 8. Irreversible Inhibition of Mammalian *S*-Adenosyl-L-methionine Decarboxylase by Substrate Analogues

Marvin Pankaskie and Mahmoud M. Abdel-Monem*

College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455. *Received August 6, 1979*

Analogues of *S*-adenosyl-L-methionine (**1**) were synthesized and evaluated as inhibitors of the enzyme *S*-adenosyl-L-methionine decarboxylase from rat liver. The compounds synthesized were *S*-(5′-deoxy-5′-adenosyl)-(±)-2-methylhomocysteine (**10**), *S*-(5′-deoxy-5′-adenosyl)-(±)-2-methylmethionine dihydrogen sulfate (**11**), *S*-(5′-deoxy-5′-adenosyl)-(±)-2-methylhomocysteine sulfoxide (**12**), *S*-(5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine hydrogen sulfate (**16**), *S*-(5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-(methylthio)propylamine dihydrogen sulfate (**17**), *S*-(5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine sulfoxide hydrogen sulfate (**18**), *N*-(cyanoethyl)-*N*-methyl-5′-amino-5′-deoxyadenosine (**20**), and *N*-(aminopropyl)-*N*-methyl-5′-amino-5′-deoxyadenosine dihydrochloride (**21**). *S*-Adenosyl-L-methionine decarboxylase was partially purified from rat liver homogenate using a methylglyoxal bis(guanylhydrazone) linked Sepharose column in the final purification step. At the highest concentration used (2.5×10^{-4} M), compounds **10**, **12**, **16**, **18**, and **20** did not produce inhibition of the enzymatic decarboxylation of [^{14}C]carboxyl-labeled *S*-adenosyl-L-methionine. Compounds **11**, **17**, and **21** were competitive inhibitors of *S*-adenosyl-L-methionine decarboxylase, and the apparent K_i values for these compounds were calculated to be 1.8×10^{-4}, 1.2×10^{-4}, and 1.1×10^{-4} M, respectively. Compounds **11** and **17** formed azomethine bonds with an essential carbonyl group in the enzyme active site which was reducible with sodium cyanoborohydride. These two inhibitors also caused a time-dependent inactivation of the enzyme, which was also dependent on the concentration of the inhibitor in the incubation media. Compound **21** did not form an azomethine bond with the enzyme and did not cause inactivation of the enzyme. These results suggest that the sulfonium analogues **11** and **17** have a binding mode to the enzyme active site which is different than that for the nitrogen analogue **21**.

The enzyme *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) is an essential enzyme in the biosynthesis of the polyamines spermidine and spermine. It catalyzes the decarboxylation of *S*-adenosyl-L-methionine (**1**) to give *S*-(5′-deoxy-5′-adenosyl)-1-(methylthio)propylamine (**2**). The enzyme spermidine synthase catalyzes the transfer of the aminopropyl moiety from **2** to putrescine to give spermidine. Similarly, the enzyme spermine synthase catalyzes the formation of spermine from **2** and spermidine. Recently, the enzyme *S*-adenosyl-L-methionine decarboxylase was purified to homogeneity from mammalian tissues,[1,2] yeast,[3] and bacteria.[4] A pyruvyl residue was identified as the prosthetic group essential for the catalytic activity of the enzymes from these three sources.[1-4] Only two other amino acid decarboxylases are known to contain covalently bound pyruvate as the catalytically essential carbonyl group in place of the commonly present pyridoxal 5′-phosphate.[5]

Methylglyoxal bis(guanylhydrazone) (**3**) and methylglyoxal bis(aminoguanylhydrazone) (**4**) are powerful inhibitors of the mammalian and yeast *S*-adenosyl-L-methionine decarboxylases. Compound **3** produced readily

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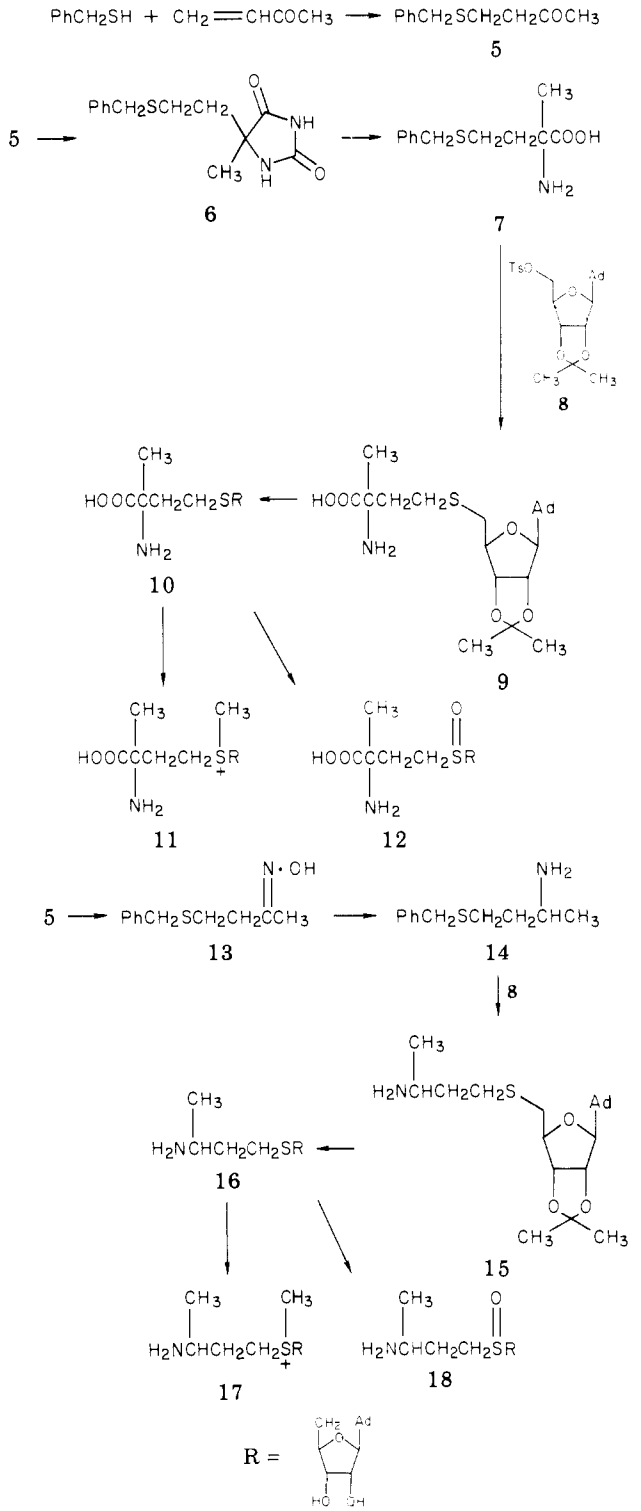
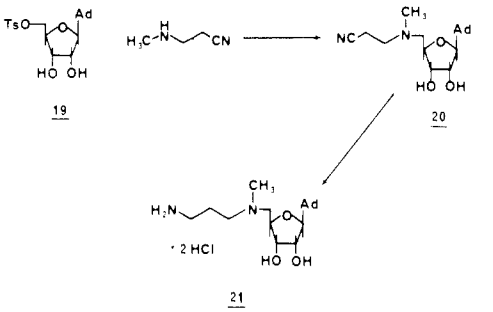
Scheme I



Scheme II



reversible competitive inhibition of the enzyme. The inhibition by **4** was initially competitive, in short-term incubation studies, but was irreversible on extended incubations.[6] These two inhibitors were used for the modulation of polyamine levels in tissues and cells in many studies. However, there are many disadvantages associated with the use of these inhibitors in studies of the function of polyamines. The effects of **3** on cell growth, even at micromolar concentrations, may not be related solely to its effect on *S*-adenosyl-L-methionine decarboxylase and on polyamine biosynthesis.[7-9] Furthermore, compound **3** caused paradoxical increases in polyamine levels and *S*-adenosyl-L-methionine decarboxylase activities in vivo.[10] Structural modifications of **3** usually resulted in the loss of *S*-adenosyl-L-methionine decarboxylase inhibitory activity.[11,12] Therefore, we initiated studies to investigate the potential utility of some *S*-adenosyl-L-methionine analogues as specific inhibitors of *S*-adenosyl-L-methionine decarboxylase. Very little is known about the structural requirements for binding of the substrate, and its analogues, to mammalian *S*-adenosyl-L-methionine decarboxylase. It was of interest to systematically modify the structure of *S*-adenosyl-L-methionine and assess the effects of these modifications on the ability of the ligand to bind to the enzyme. In this report, we describe the synthesis of substrate analogues in which the sulfonium group and the substituents of the α carbon of the amino acid moiety have been modified. The evaluation of these analogues as inhibitors of partially purified *S*-adenosyl-L-methionine decarboxylase from rat liver is also described.

Results and Discussion

Chemistry. The target compounds **10–12**, **16–18**, **20**, and **21** were obtained as shown in Schemes I and II. 4-(Benzylthio)-2-butanone (**5**) was obtained by the addition of benzyl mercaptan to methyl vinyl ketone. Compound **5** was subjected to the Buchrer–Lieb synthesis to provide the hydantoin **6**, which was hydrolyzed with barium hydroxide to give **7**. The thiolate anion of **7** was generated by sodium in liquid ammonia and condensed with 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine (**8**) to give **9**, which was not purified but was immediately hydrolyzed in aqueous sulfuric acid to give **10**. The target compound **11** was obtained by methylation of **10** using a modification of the procedure described by Samejima et al.[13] The synthesis of **11** by a different route was recently reported by Nakamura et al.[14] 2-Amino-4-(benzylthio)-butanone (**14**) was obtained by lithium aluminum hydride reduction of the oxime **13**. The latter was obtained by the condensation of hydroxylamine with **5**. Compound **15** was obtained by the condensation of the thiolate anion of **14** with **8**, followed by acid hydrolysis. Methylation of **16** with

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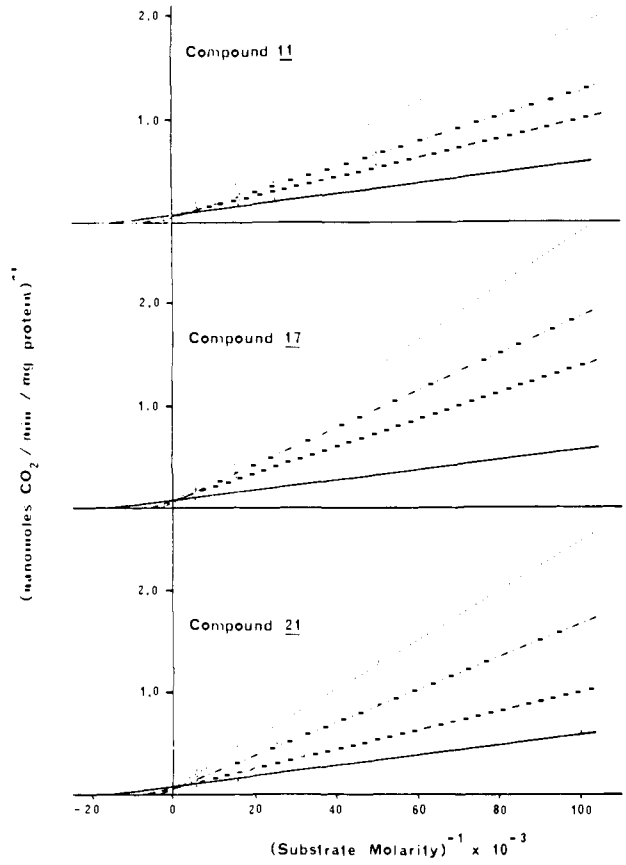

Figure 1. Double-reciprocal plots of the effect of compounds 11, 17, and 21, respectively, on the rate of decarboxylation of *S*-adenosyl-L-methionine by *S*-adenosyl-L-methionine decarboxylase. Concentrations of inhibitors used were: control (—), 6.25×10^{-5} M (- - -), 1.25×10^{-4} M (-•-), and 2.5×10^{-4} M (·····).

methyl iodide in the presence of silver acetate provided **17**. Oxidation of **10** and **16** with hydrogen peroxide in acetic acid provided **12** and **18**, respectively. The condensation of *N*-methyl-β-alaninenitrile with 5′-*O*-toluene-*p*-sulfonyladenosine (**19**) gave, after purification with column chromatography, **20**. Reduction of **20** with diborane provided compound **21** in good yields.

Enzyme Inhibition Studies. The inhibition by the target compounds of the decarboxylation of *S*-adenosyl-L-methionine by a partially purified enzyme from rat liver was studied in vitro. The enzyme, *S*-adenosyl-L-methionine decarboxylase, was partially purified from the 105000*g* supernatant from rat liver homogenate using a minor modification of the procedure developed by Pegg.[15] In this procedure, a methylglyoxal bis(guanylhydrazone) linked Sepharose column was used in the final purification step. The decarboxylase activity was measured by determining the amount of $^{14}CO_2$ released from [^{14}C]-carboxyl-labeled *S*-adenosyl-L-methionine using the procedure of Pegg and Williams-Ashman.[16] Under the assay conditions, *S*-adenosyl-L-methionine decarboxylase from rat liver had an apparent K_m for *S*-adenosyl-L-methionine of 5.14×10^{-5} M and a V_{max} of 8.72 nmol of CO_2/min^{-1} (mg of protein)$^{-1}$. The target compounds were tested at concentrations of 6.25×10^{-5}, 1.25×10^{-4}, and 2.5×10^{-4} M and substrate concentrations ranging between 2.0×10^{-5} and 1.6×10^{-4} M. At the highest concentrations tested, compounds **10**, **12**, **16**, **18**, and **20** did not suppress the enzymatic production of $^{14}CO_2$ from [^{14}C]carboxyl-labeled

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Table I. Effects of Preincubation with Different Inhibitors on the Recovery of *S*-Adenosyl-L-methionine Decarboxylase Activity

additions to incubation media	% act. recovered after[a] 40 min	80 min
none	100[b]	100[c]
0.2 mM 1	69 ± 6[d]	43 ± 4
0.2 mM 1 + 1 mM $NaBH_3CN$	17 ± 2	nd
0.2 mM 11	89 ± 2	81 ± 4
0.4 mM 11	77 ± 5	62 ± 2
0.2 mM 11 + 1 mM $NaBH_3CN$	50 ± 4	nd
0.2 mM 17	75 ± 2	66 ± 6
0.4 mM 17	65 ± 2	42 ± 1
0.2 mM 17 + 1 mM $NaBH_3CN$	21 ± 1	nd
0.2 mM 21	95 ± 4	97 ± 1
0.4 mM 21	93 ± 2	97 ± 5
0.2 mM 21 + 1 mM $NaBH_3CN$	94 ± 2	nd

[a] The enzymatic activity recovered in the absence of any additions was assigned a value of 100 and the activity of other samples was reported as a percentage of that. [b] Incubation of the enzyme with the buffer at 37 °C for 40 min resulted in the recovery of 86% of the activity at 0 min. [c] Incubation of the enzyme with the buffer at 37 °C for 80 min resulted in the recovery of 76% of the activity at 0 min. [d] Each value is the mean (plus or minus the coefficient of variation).

S-adenosyl-L-methionine. However, compounds **11**, **17**, and **21** produced a significant inhibition of the enzymatic decarboxylation of *S*-adenosyl-L-methionine by the rat liver *S*-adenosyl-L-methionine decarboxylase. Examination of the Lineweaver–Burk reciprocal plots (Figure 1) of the data indicates that these compounds are competitive inhibitors of the enzymatic decarboxylation reaction.[17] The apparent K_i values for compounds **11**, **17**, and **21** were calculated to be 1.8×10^{-4}, 1.2×10^{-4}, and 1.1×10^{-4} M, respectively. It should be pointed out that **11** may be a substrate for the enzyme and that the apparent competitive inhibition observed with this compound may be due to its ability to act as an alternate substrate. Furthermore, compounds **11** and **17** are actually mixtures of four isomers. Since the decarboxylase inhibitory activity may reside in only one of the isomers, the intrinsic activity of these structural analogues may be higher than the observed values.

Previously, we reported that the substrate and/or the decarboxylated product formed an azomethine bond with the pyruvate cofactor which can be reduced efficiently with sodium cyanoborohydride.[12] We also reported that incubation of the enzyme with substrate resulted in a time-dependent irreversible inactivation of the enzyme. This inactivation was not reversed by extensive dialysis, and the use of *S*-[^{3}H]methyl-labeled substrate did not result in the incorporation of radioactivity in the dialyzed enzyme. It was of interest, therefore, to determine if the target compounds form azomethine bonds with the pyruvate residue in the enzyme active site and if they will also produce irreversible inhibition of the enzyme. Azomethine compounds such as **22** could be trapped in the presence of free pyruvate residues by selective reduction with cyanoborohydride, and the presence of such intermediates in an incubation media containing the enzyme and **11**, **17**, or **21**

(17) Compounds 11 and 17 were later found to produce time-dependent irreversible inhibition of the enzyme. The rate equations which describe this type of inhibition are different than those which describe reversible inhibition. A complete study of the kinetics of inactivation of the enzyme by 11 and 17 is underway. The apparent K_i values, calculated from relatively short-term incubations, are included only to provide an estimate of the relative inhibitory activities of the different analogues studied.

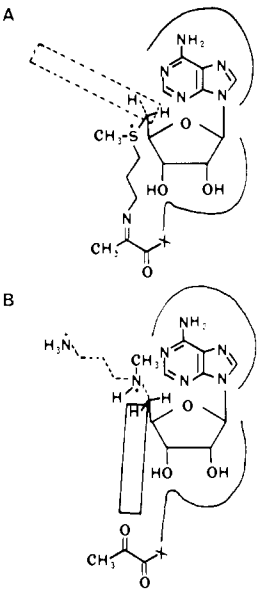


Figure 2. Proposed binding modes to the active site of *S*-adenosyl-L-methionine decarboxylase by the sulfonium analogues (A) and nitrogen analogue of *S*-adenosyl-L-methionine.

could be demonstrated if a loss of enzymatic activity is observed on treatment of the mixture with sodium cyanoborohydride.

Compounds **11**, **17**, and **21** at 0.2 and 0.4 M concentrations were incubated with the partially purified enzyme at 37 °C in the presence and absence of 1 mM sodium cyanoborohydride. Aliquots of the incubation mixtures were diluted and the remaining decarboxylase activity was measured at 0, 40, and 80 min. The results of these experiments are shown in Table I. From these results it appears that **11** and **17** form azomethine bonds with the enzyme which are reducible with sodium cyanoborohydride. Furthermore, these two inhibitors caused a time-dependent inactivation of the enzyme, which was also dependent on the concentration of inhibitor in the incubation media. On the other hand, **21** does not appear to form an azomethine bond with the enzyme, since the addition of sodium cyanoborohydride did not result in significant loss of enzymatic activity. This inhibitor does not appear to cause inactivation of the enzyme similar to that observed with **11** and **17**. The inactivation of the enzyme produced by **11** and **17** in the presence or absence of sodium cyanoborohydride was not reversed by dialysis. It is noteworthy that the substrate and the inhibitors which form azomethine bonds with the enzyme also caused an irreversible loss of catalytic activity, while the inhibitor which did not form an azomethine bond did not cause enzyme inactivation. These observations indicate that the binding mode for the sulfonium ligands **11**, **17**, and **1** is different than that for the nitrogen ligand **21**. This is represented schematically in Figure 2. It is proposed that both the sulfonium ligands and the nitrogen ligand bind to the same sites which recognize the adenine and ribosyl moieties of the substrate. However, the sulfonium group binds to a recognition site and juxtaposition the amino acid chain and the pyruvyl group to form the azomethine bond, as shown in Figure 2A. The diaminopropyl moiety of compound **21** appears to bind away from the pyruvyl group. It must be emphasized that the primary amino group in **21** must be essential for binding, since compound **20** was not a competitive inhibitor of the enzymatic reaction.

The above results provide some information regarding the requirements for binding of the substrate, and its analogues, to *S*-adenosyl-L-methionine decarboxylase. It appears that the carboxyl group of the amino acid portion of the substrate is not essential for binding, as demonstrated by the activity of **17** and **21** which do not have an α-carboxyl group. The approximately equal apparent K_i values for **11** and **17** suggest that the contribution of the carboxyl group to binding, if any, is very small. However, the equal K_i values should not be construed to indicate that the carboxyl group does not contribute to binding. Both **11** and **17** are mixtures of isomers, and it is expected that the inhibitory activity will reside in only one of the isomers for each compound. It is possible that the degree of stereoselectivity of the enzyme to the isomers of **11** is greater than that for the isomers of **17**, and the apparent K_i for **17** represents a greater contribution of the less active isomers.

Replacement of the α hydrogen of the substrate with a methyl group does not seem to prevent the binding of the ligand to the enzyme. Previous studies with pyridoxal phosphate dependent decarboxylases indicated that substitution of the α hydrogen in the substrate with a methyl group gave analogues which have greater affinity for the enzyme than the substrate. Many of these α-methyl analogues of amino acids produced inhibition of the respective decarboxylases, partially by acting as alternative substrates. It appears, however, that with *S*-adenosyl-L-methionine decarboxylase, a pyruvate-dependent enzyme, the replacement of the α hydrogen with a methyl group did not enhance the affinity of the analogue for binding to the enzyme. It will be of interest to determine if **11** is a substrate for the enzyme.

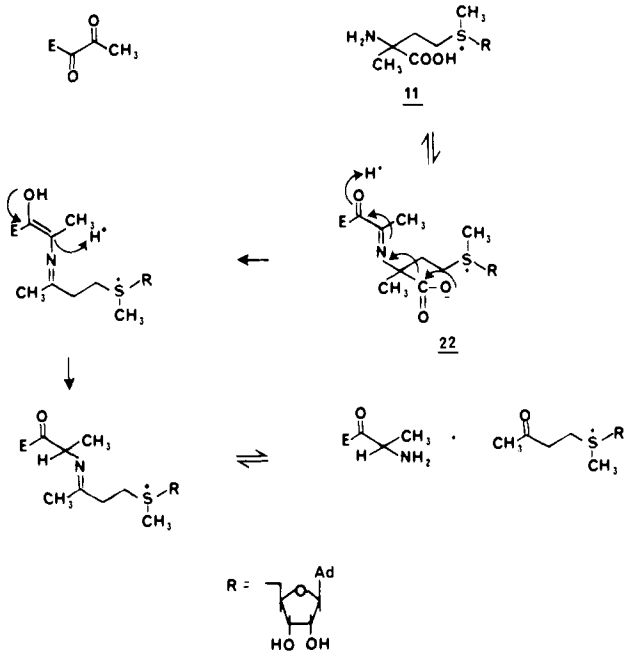
The α-amino group appears to be essential for binding to the enzyme, as demonstrated by the lack of inhibitory activity of **20**. However, it is not known if replacement of the α-amino group with another cationic group, such as hydrazino or guanidino, would produce an active inhibitor. As will be discussed later, the α-amino group appears to play two different roles, depending on the binding mode of the ligand to the enzyme.

The sulfonium group in the substrate is not an absolute requirement for binding. Replacement of the sulfonium group with nitrogen in **21** provided an active inhibitor. It appears, however, that a positively charged group at the position of the sulfonium group is essential for binding. This is demonstrated by the lack of activity of the thioethers **10** and **16** and the sulfoxides **12** and **18**. The nitrogen analogue **21** would be present predominantly in the protonated form at physiological pH and probably it is the protonated form which binds to the enzyme.

A very significant finding in this work is the observation that **11**, **17**, and the natural substrate (**1**) produced irreversible inactivation of the enzyme. The exact mechanism of this irreversible inhibition is not known and is currently under investigation in our laboratory. However, the specificity of the irreversible inhibition to the sulfonium ligands suggests that one of the following is the most likely mechanism of inactivation. First, it is possible that the binding of the natural substrate (**1**), or inhibitors **11** and **17**, will cause conformational changes in the enzyme which make the protein more labile to enzymatic or nonenzymatic degradation. The enzyme preparations used in these studies were only partially purified and may have contained proteases which hydrolyzed the enzyme after it has undergone ligand-induced conformational changes.

Secondly, the irreversible inhibition of *S*-adenosyl-L-methionine decarboxylase by the sulfonium ligands may be a direct consequence of the formation of the azomethine bond between the amino group of the inhibitor and the

Scheme III



covalently bound essential carbonyl group in the enzyme active site. This proposed mechanism is shown in Scheme III for 11. The enzyme may catalyze the decarboxylation-dependent transamination of 11, as has been shown with a number of pyridoxal-dependent decarboxylases.[18,19] The transamination reaction would result in transforming the covalently bound pyruvyl prosthetic group to an alanyl residue and the loss of catalytic activity. Decarboxylation-dependent transamination with pyridoxal-dependent decarboxylases resulted in the formation of pyridoxylamine phosphate from pyridoxal phosphate and depletion of the cofactor.

While this proposed mechanism is admittedly speculation at this time, it is compatible with our experimental results and is based on acceptable chemical principles. The irreversible inhibition of S-adenosyl-L-methionine decarboxylase by 11, 17, and the substrate is of great biological significance regardless of the exact mechanism of inhibition. This observation should provide better understanding of the control mechanism of the enzymatic activity in vivo.

Experimental Section

The melting points were determined in open capillary tubes and are uncorrected. Elemental analysis was performed by M-H-W Laboratories, Phoenix, Ariz. The IR spectra were obtained with a Perkin-Elmer 237 B or Perkin-Elmer 281 spectrophotometer. The NMR spectra were taken on a Varian A-60D in $CDCl_3$ or D_2O with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standard, respectively. Mass spectral analyses were performed on AEI MS-30 at 20–70 eV and 25–200 °C chamber temperature. All spectral data were consistent with the proposed structures. Radioactivity was measured using a Beckman LS-100C liquid scintillation counter.

4-(Benzylthio)-2-butanone (5). This reaction should be carried out in a well-ventilated fume hood! To methyl vinyl ketone (14.0 g, 0.2 mol) at 25 °C was added benzyl mercaptan (24.8 g, 0.2 mol) and five drops of piperidine. The reaction mixture was stirred for 2–3 h, diluted with ether (100 mL), and extracted with 150 mL each of 5% hydrochloric acid, water, and saturated sodium chloride solution. The ether phase was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was distilled in vacuo to give **5** (32.5 g, 84%), bp 100–103 °C (0.1 mmHg). Anal. ($C_{11}H_{14}OS$) C, H, O.

5-[3-(Thioethyl)benzyl]-5-methylhydantoin (6). A solution of $NaHSO_3$ (5.2 g, 0.05 mol) in water (50 mL) was added slowly to a solution of **5** (9.7 g, 0.05 mol) in ethanol (50 mL). The mixture was stirred at room temperature for 30 min and then treated with KCN (6.5 g, 0.1 mol) and ammonium carbonate (20.0 g, 0.2 mol). The mixture was heated at 50–55 °C overnight and concentrated to 25 mL. The solution was cooled and the precipitated solid filtered to give **6** (11.5 g, 88%), mp 149–150 °C. An analytical sample was obtained by recrystallization from aqueous ethanol. Anal. ($C_{13}H_{16}N_2O_2S$) C, H, N.

(±)-2-Amino-4-(benzylthio)-2-methylbutanoic Acid (7). To a suspension of **6** (10.4 g, 0.04 mol) in water (150 mL) was added barium hydroxide (35 g, 0.11 mol), and the mixture was stirred overnight at 50–55 °C. The reaction mixture was cooled and filtered, and carbon dioxide gas was bubbled through the clear filtrate for 30 min. The mixture was filtered, and carbon dioxide gas was again bubbled through the clear filtrate. This was repeated until no more precipitate formed after treatment with carbon dioxide gas. The clear solution was concentrated until a slight turbidity was observed and then allowed to stand at 4 °C overnight. The precipitated solids were filtered and dried in vacuo to give **7** (7.8 g, 85%), mp 252–254 °C. An analytical sample was obtained by recrystallization from water. Anal. ($C_{12}H_{17}NO_2S$) C, H, N.

***S*-(5′-Deoxy-5′-adenosyl)-(±)-2-methylhomocysteine (10).** Sodium metal was added to a solution of **7** (1.9 g, 7.9 mmol) in liquid ammonia (100 mL) until a blue color persisted for 15 min. 2′,3′-*O*-Isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine (**8**; 3.7 g, 8.0 mmol) was then added to the solution and the ammonia allowed to evaporate at room temperature overnight. The residue was dissolved in water (25 mL) and adjusted to pH 7 with 5% hydrochloric acid. The clear solution was extracted with methylene chloride (2 × 50 mL) and the aqueous layer lyophilized. The residue was dissolved in a minimal amount of water and applied to a column of cellulose (30–35 g dry weight of Whatman CF 11 cellulose), and the product was eluted with ethanol/water (3:1). Fractions were collected and monitored by TLC [Avicel F, ethanol/water (3:2)], and those containing the desired product were pooled and evaporated in vacuo to give *S*-(2′,3′-*O*-isopropylidene-5′-deoxy-5′-adenosyl)-(±)-2-methylhomocysteine (**9**). This intermediate was not further purified but dissolved in 1 N sulfuric acid (25 mL) and allowed to stand at room temperature for 48 h. The solution was adjusted to pH 6–7 by the addition of solid barium carbonate and filtered twice, and the filtrate was lyophilized. The residue was dissolved in water (1–2 mL) and treated with ethanol to a distinct turbidity. After the solution was left standing at 4 °C for several days, the solid precipitate was filtered and dried in vacuo to give **10** (1.4 g, 51%) as a slightly hygroscopic solid, mp 243 °C dec. The product was homogeneous by TLC [silica gel GF, butanol/acetic acid/water (12:3:5)]: IR (KBr) 3220, 2950, 2510, 1640, 1600, 1515, 1480, 1400, 1370, 1330, 1305, 1250, 1210, 1120, 1085, 1040, 790 cm^{-1}; NMR (D_2O) δ 8.2 (s, 1 H), 8.0 (s, 1 H), 5.9 (d, 1 H, J = 2.5 Hz), 4.8–4.2 (m, 3 H), 2.8–2.0 (m, 4 H), 3.0 (m, 2 H), 1.43 and 1.37 (2 s, 3 H). Anal. ($C_{15}H_{22}N_6O_5S$) C, H, N.

***S*-(5′-Deoxy-5′-adenosyl)-(±)-2-methylmethionine Dihydrogen Sulfate (11).** Silver acetate (150 mg, 1.0 mmol) and iodomethane (0.2 mL, 25 mmol) were added to a solution of **10** (200 mg, 0.5 mmol) in acetic acid/formic acid (1:1). The solution was mixed well and allowed to stand at room temperature for 3–4 h. The solution was centrifuged to remove the precipitated AgI, treated with an equal volume of water, and extracted with ether (3 × 25 mL). The aqueous layer was treated with 1–2 mL of 1 N sulfuric acid and lyophilized. The residue was dissolved in 1–2 mL of 1 N sulfuric acid and treated with absolute ethanol (30 mL). After the solution was left standing at 4 °C overnight, an oily precipitate formed which solidified in absolute ethanol. The solids were collected and dried in vacuo to give 11 (110 mg, 68%): mp 155 °C dec; IR (KBr) 3375, 2510, 1720, 1685, 1600, 1500, 1415, 1210, 1180, 1100, 1030, 840 cm^{-1}; NMR (D_2O) δ 8.2 (s, 1 H), 8.0 (s, 1 H), 5.9 (d, 1 H, J = 2.5 Hz), 4.8–4.2 (m, 3 H), 2.8–2.0 (m, 4 H), 3.1 (2 s, 3 H), 3.0 (m, 2 H), 1.43 and 1.37 (2 s, 3 H). Anal. ($C_{16}H_{26}N_6O_5S \cdot 2HSO_4$) C, H, N.

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(19) M. H. O'Leary and R. L. Banghin, *J. Biol. Chem.*, **252**, 768 (1977).

4-(Benzylthio)-2-butanone Oxime (13). To a solution of **5** (9.7 g, 50 mmol) in 5% aqueous Na_2CO_3 (100 mL) was added, with vigorous stirring, $NH_2OH{\cdot}HCl$ (5.25 g, 75 mmol), and the mixture was stirred overnight at room temperature. The solution was extracted with methylene chloride (2 × 100 mL) and the organic layer evaporated in vacuo. The residual oil was distilled in vacuo to give **13** (5.5 g, 52%), bp 148–150 °C (0.005 mmHg). Anal. ($C_{11}H_{15}NOS$) C, H, N.

2-Amino-4-(benzylthio)butane (14). To a stirred suspension of $LiAlH_4$ (0.4 g, 10 mmol) in dry ether (100 mL) under a N_2 atmosphere was added **13** (1.0 g, 5 mmol), and the solution was heated to reflex for 3 h. After the reaction had cooled to room temperature, the ethereal solution was treated with 10% sodium hydroxide solution to decompose excess reducing agent. The organic layer was separated, washed with water (50 mL), and evaporated in vacuo. The residual oil was distilled in vacuo to give **14** (732 mg, 73%), bp 109–111 °C (0.005 mmHg). Anal. ($C_{11}H_{17}NS$) C, H, N.

***S*-(5′-Deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine Hydrogen Sulfate (16).** Sodium metal was added to a solution of **14** (2.0 g, 10 mmol) in liquid ammonia (100 mL) until a blue color persisted for 15 min. 2′,3′-*O*-Isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine (**8**; 4.6 g, 12 mmol) was then added to the solution, and the ammonia was allowed to evaporate at room temperature overnight. The residue was dissolved in 1 N sulfuric acid (50 mL) and extracted with methylene chloride (50 mL). The organic layer was extracted with an additional 50 mL of 1 N sulfuric acid, and the aqueous layers were pooled. The solution was adjusted to pH 10 with concentrated ammonium hydroxide and extracted with methylene chloride (3 × 50 mL), and the organic phase was evaporated in vacuo to give *S*-(2′,3′-*O*-isopropylidene-5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine hydrogen sulfate (**15**). This intermediate was not further purified but was dissolved in 20 mL of 1 N sulfuric acid and allowed to stand at room temperature for 48 h. Ethanol was added to a distinct turbidity and the solution was allowed to stand for several days at 4 °C. The solid precipitate formed was collected and dried in vacuo to give **16** (2.8 g, 63%): mp 196–198 °C dec; IR (KBr) 3350, 3100, 2920, 1695, 1610, 1500, 1415, 1215, 1110, 1050, 710 cm^{-1}; NMR (D_2O) δ 8.21 (s, 1 H), 8.18 (s, 1 H), 5.90 (d, 1 H, J = 2.0 Hz), 4.8–4.2 (m, 3 H), 3.25 (m, 1 H), 2.82 (m, 2 H), 2.41 (m, 2 H), 1.85 (m, 2 H), 1.08 (d, 3 H, J = 3.0 Hz). Anal. ($C_{14}H_{22}N_6O_3S{\cdot}H_2SO_4$) C, H, N.

***S*-(5′-Deoxy-*S*′-adenosyl)-(±)-1-methyl-3-(methylthio)-propylamine Dihydrogen Sulfate (17).** Silver acetate (75 mg, 0.45 mmol) and iodomethane (1.5 mL, 20 mmol) were added to a solution of **16** (100 mg, 0.22 mmol) in acetic acid/formic acid (1:1). The solution was mixed well and allowed to stand at room temperature for 3–4 h. The solution was centrifuged to remove precipitated AgI, treated with an equal volume of water, and extracted with ether (3 × 25 mL). To the clear aqueous layer was added 1 N sulfuric acid (1–2 mL), and the solution was lyophilized. To the residue, dissolved in 1 N sulfuric acid (1–2 mL), was added ethanol (30 mL), and the solution was allowed to stand overnight at 4 °C. The solids were collected and dried in vacuo to give **17** (63 mg, 60%): mp 165 °C dec; IR (KBr) 3380, 1690, 1610, 1510, 1425, 1220, 1180, 1105, 1040, 840 cm^{-1}; NMR (D_2O) δ 8.2 (s, 1 H), 8.16 (s, 1 H), 5.9 (d, 1 H, J = 2.0 Hz), 4.8–4.2 (m, 3 H), 3.25 (m, 1 H), 3.1 (2 s, 3 H), 2.8 (m, 2 H), 2.4 (m, 2 H), 2.4 (m, 2 H), 1.85 (m, 2 H), 1.1 (d, 3 H, J = 3.0 Hz). Anal. ($C_{15}H_{26}N_6O_3S{\cdot}2HSO_4{\cdot}0.5C_2H_5OH$) C, H, N.

***S*-(5′-Deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine Sulfoxide Hydrogen Sulfate (18).** To a suspension of **16** (200 mg, 0.44 mmol) in glacial acetic acid (2 mL) was added 30% aqueous hydrogen peroxide (0.25 mL, 2 mmol), and the solution was stirred overnight at room temperature. Addition of ethanol precipitated a white solid, which after recrystallization from aqueous ethanol gave **18** (150 mg, 73%): mp 190 °C dec; IR (KBr) 3100 (br), 2960, 1690, 1610, 1505, 1400, 1285, 1220, 1120, 1100, 1050 cm^{-1}. Anal. ($C_{14}H_{22}N_6O_4S{\cdot}H_2SO_4{\cdot}H_2O$) C, H, O.

***S*-(5′-Deoxy-5′-adenosyl)-(±)-2-methylhomocysteine Sulfoxide (12).** To a suspension of **10** (200 mg, 0.46 mmol) in glacial acetic acid (2 mL) was added 30% aqueous hydrogen peroxide (0.25 mL, 2 mmol), and the solution was stirred overnight at room temperature. Addition of ethanol precipitated a white solid, which after recrystallization from water gave **12** (185 mg, 88%): mp 150 °C dec; IR (KBr) 3180 (br), 1650, 1600, 1490, 1400, 1170, 1120, 1040, 1010, 810, 680 cm^{-1}. Anal. ($C_{15}H_{22}N_6O_6S{\cdot}H_2O$) C, H, N.

2′,3′-*O*-Isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine (8). This compound was prepared according to the method of Sakami[20] and stored over P_2O_5 until needed.

5′-*O*-Toluene-*p*-sulfonyladenosine (19). This intermediate was prepared according to the method of Schmidt et al.,[21] mp 152–154 °C (lit. mp 154 °C).

***N*-(Cyanoethyl)-*N*-methyl-5′-amino-5′-deoxyadenosine (20).** A suspension of **19** (1.5 g, 3.5 mmol) in *N*-methyl-β-alaninenitrile (10 mL) was allowed to stand at room temperature for 5 days. The reaction mixture was then poured into ether (200 mL), and the resulting oily residue was isolated by decantation of the ether layer. This oil was taken up in a mixture of ethanol/methylene chloride (1:9), applied to a column of silica gel (10 g), and eluted with a mixture of ethanol/methylene chloride/ammonium hydroxide (1:9:0.1). Fractions containing the desired product, as determined by TLC (silica gel GF; ethanol/methylene chloride/ammonium hydroxide, 3:16:1) were pooled, evaporated in vacuo, and recrystallized from absolute ethanol to give **20** (950 mg, 80%): mp 151 °C; IR (KBr) 3410, 3320, 3170, 3110, 2820, 2240, 1670, 1610, 1570, 1475, 1410, 1330, 1250, 1200, 1120, 1045, 950, 810, 710 cm^{-1}; NMR (Me_2SO-d_6) δ 8.0 (s, 1 H), 7.9 (s, 1 H), 5.17 (d, 1 H, J = 2.5 Hz), 4.5 (m, 1 H), 4.0 (m, 4 H), 2.4 (m, 6 H), 2.1 (s, 3 H). Anal. ($C_{14}H_{19}N_7O_3$) C, H, N.

***N*-(Aminopropyl)-*N*-methyl-5′-amino-5′-deoxyadenosine Dihydrochloride (21).** To a stirred solution of **20** (100 mg, 0.3 mmol) in THF (20 mL) under an atmosphere of N_2 was added a solution of BH_3/THF (0.5 mL, 0.5 mmol), and the reaction was allowed to stir for 3 h. An equal volume of absolute ethanol was added to decompose excess reducing agent, followed by an equal volume of ethanolic hydrochloride acid. The solution was evaporated in vacuo, and to the residue was added absolute methanol (30 mL). After evaporation of the solution in vacuo, the residue was recrystallized from 95% ethanol to give **20** (75 mg, 75%): mp 160 °C dec; IR (KBr) 3300, 2960, 1690, 1600, 1510, 1420, 1320, 1215, 1125, 1040, 810 cm^{-1}; NMR (D_2O) δ 8.0 (s, 1 H), 7.9 (s, 1 H), 5.7 (s, 1 H), 4.5–4.0 (m, 3 H), 2.6–2.0 (m, 8 H), 2.1 (s, 3 H). Anal. ($C_{14}H_{23}N_7O_3{\cdot}2HCl$) C, H, N.

Enzyme Purification. Rat liver *S*-adenosyl-L-methionine decarboxylase was purified by the method of Pegg[14] with very slight modifications. Twelve male Sprague–Dawley rats (250 g) were injected intraperitoneally with methylglyoxal bis(guanylhydrazone) (**3**; 80 mg/kg) and 24 h later were sacrificed by cervical dislocation. The livers were removed and homogenized in 200 mL of buffer A (containing 10 mM Tris-HCl, pH 7.5; 2.5 mM putrescine; 1 mM dithiothreitol; and 0.1 mM EDTA) at 4 °C. All subsequent operations were carried out at 0–5 °C. The homogenate was centrifuged at 105000*g* for 90 min. The supernatant was treated with $(NH_4)_2SO_4$, and the protein fraction precipitating between 35 and 65% saturation was collected by centrifugation. The protein pellet was dissolved in the minimum amount of buffer A, and the solution was dialyzed overnight against 4 L of the same buffer.

A glass column (2.5 × 30 cm) was packed with Sepharose linked to methylglyoxal bis(guanylhydrazone) and washed with 200 mL of buffer A. The dialyzed protein sample was applied to the column at a flow rate of 35–40 mL/h, and the column was washed with 100 mL of buffer A, followed by 150 mL of buffer A containing 0.3 M NaCl. Protein having *S*-adenosyl-L-methionine decarboxylase activity was eluted off the column with buffer A containing 0.3 N NaCl and 1 mM methylglyoxal bis(guanylhydrazone). The column eluants were collected in 10-mL fractions, and each of these fractions was assayed for decarboxylase activity using 50 μL in the standard assay procedure. Decarboxylase activity was found in fractions 4–6, with more than 80% of the recovered activity in fraction 5 alone. The presence of methylglyoxal bis(guanylhydrazone) in the elution solvent greatly reduced the apparent activity of these fractions. Fractions containing decarboxylase activity were pooled, divided into 5-mL aliquots, and stored at 0–5 °C. The protein solution was dialyzed against

(20) W. Sakami, *Biochem. Prep.*, **8**, 6 (1958).
(21) R. R. Schmidt, U. Schloz, and D. Schwille, *Chem. Ber.*, **101**, 590 (1968).

two changes of 1 L each of buffer A, to remove the inhibitor, immediately before use as a source of the decarboxylase enzyme. Using this method, an enzyme preparation having a specific activity of about 15 nmol of CO_2 min^{-1} (mg of protein)$^{-1}$ was routinely obtained.

Assay of *S*-Adenosyl-L-methionine Decarboxylase Activity. *S*-Adenosyl-L-methionine decarboxylase activity was determined by a modification of the method of Pegg and Williams-Ashman.[16] The standard assay medium contained 0.1 M sodium phosphate buffer (pH 7.0), 2.5 mM dithiothreitol, 2.5 mM putrescine, 0.2 mM *S*-[1-^{14}C]adenosyl-L-methionine (2.0×10^5 cpm), and the enzyme preparation (50 μL) in a total volume of 1.0 mL. Reactions were carried out in 16 × 100 mm test tubes sealed with rubber stoppers carrying a polypropylene well containing 0.3 mL of ethanolamine. After incubation for 30 min at 37 °C, the reaction was terminated and $^{14}CO_2$ released by the injection of 0.5 mL of 5 N H_2SO_4 through the rubber stopper. The tubes were incubated for an additional 45 min at 25 °C, and the well and its contents were transferred into a glass scintillation vial containing 10 mL of scintillation cocktail. The samples were stored in the dark overnight and counted. All counts were corrected for the nonenzymatic production of $^{14}CO_2$, which was determined by replacing the enzyme fraction in the incubation medium with an equal volume of buffer A. This method gave quantitative recovery of the $^{14}CO_2$ released from sodium [^{14}C]-bicarbonate.

Enzyme Inhibition Studies. The incubation media contained 0.1 M sodium phosphate buffer, pH 7.0; 2.5 mM dithiothreitol; 2.5 mM putrescine; *S*-[1-^{14}C]adenosyl-L-methionine (2.0×10^5 cpm); *S*-adenosyl-L-methionine at concentrations of 2.0×10^{-6}, 4.0×10^{-5}, 8.0×10^{-5}, and 1.60×10^{-4} M; the inhibitor at concentrations of 6.25×10^{-5}, 1.25×10^{-4}, and 2.50×10^{-4} M; and 0.1 mL of the enzyme preparation in a total volume of 1.0 mL. The incubation conditions and the measurement of $^{14}CO_2$ released were as described under "Assay of *S*-Adenosyl-L-methionine Decarboxylase Activity".

Effects on Enzymatic Activity of Preincubation with Inhibitors or Inhibitors and Sodium Cyanoborohydride. The preincubation media contained enzyme fraction in buffer A, 0.25 mL; bovine serum albumin, 0.3 mg; inhibitor at concentrations of 0.0, 0.2, or 0.4 mM; and sodium cyanoborohydride, 1.0 mM, when appropriate, in a final volume of 0.3 mL adjusted to pH 7.0. The mixtures were incubated at 37 °C, and 25-μL aliquots were removed at 0, 40, and 80 min and assayed for decarboxylase activity as described under "Assay of *S*-Adenosyl-L-methionine Decarboxylase Activity", at a substrate concentration of 0.2 mM. Under these conditions, the presence of the inhibitors and/or sodium cyanoborohydride did not change the measured enzyme activity at 0 min.

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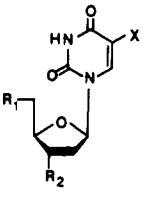
Synthesis and Biological Activities of 5-(Hydroxymethyl, azidomethyl, or aminomethyl)-2′-deoxyuridine and Related 5′-Substituted Analogues[1a]

George T. Shiau, Raymond F. Schinazi,[1b] Ming S. Chen, and William H. Prusoff*

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received August 6, 1979*

The synthesis of 5-(azidomethyl)-2′-deoxyuridine (**10**) has been accomplished by two independent methods. The first involved tosylation of 5-(hydroxymethyl)-2′-deoxyuridine (**1**) to furnish a mixture of two mono- and a ditosyl nucleosides which were converted into the corresponding 5-(azidomethyl) (**10**), 5-(azidomethyl)-5′-azido (**14**), and 5-(hydroxymethyl)-5′-azido (**15**) derivatives of 2′-deoxyuridine. The second method was more selective and required the formation of the intermediate 5-(bromomethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**8**), followed by displacement of the bromo group by lithium azide and deacetylation. Catalytic hydrogenation of the azides **9**, **10**, **14**, and **15** gave the corresponding amines **16**, **2**, **6**, and **7**, respectively. Compounds **1**, **2**, **10**, and **16** inhibited the growth of murine Sarcoma 180 and L1210 in culture, and the activity of **2** was prevented by 2′-deoxypyrimidine nucleosides but not by purine nucleosides. The replication of herpes simplex virus type 1 (HSV-1) was strongly inhibited only by **1** and **10**. Studies on the binding of the various thymidine analogues to HSV-1 encoded pyrimidine deoxyribonucleoside kinase indicate that **1** and **10** have good affinity for the enzyme.

Modifications at the C-5 position of pyrimidine deoxyribonucleosides have produced a number of compounds with selective biological activity.[2,3] Some of these derivatives are phosphorylated in cell culture with subsequent incorporation into DNA leading to a stable modified DNA. The findings that 5-(hydroxymethyl)-2′-deoxyuridine (**1**) inhibited the replication of *Escherichia coli* 15T$^-$ by Green et al.,[4] had significant cytotoxic activity on a variety of tumor cell lines by Langen and co-workers[5–7] as well as on normal cells (bovine fetal kidney) by Meldrum et al.,[8] and had antiviral activity against vaccinia and herpes simplex



	R_1	R_2	X
1	OH	OH	CH_2OH
2	OH	OH	CH_2NH_2
3	NH_2	OH	I
4	NH_2	OH	CH_3
5	OH	NH_2	CH_3

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