Analogs of S-Adenosylhomocysteine as Potential Inhibitors of Biological Transmethylation. Synthesis and Biological Activity of Homocysteine Derivatives Bridged to Adenine

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The syntheses of several acyclic analogs of S-adenosylhomocysteine and its derivatives are described by several routes. The activity of these analogs as inhibitors of the enzyme catechol-O-methyltransferase has been determined.

The general utilization of S-adenosylmethionine (SAM) as a Me donor in numerous biological reactions has been well known for many years.[1] One of our main goals in the study of nonenzymic[2] and enzymic transmethylation reactions has been the elucidation of a molecular mechanism in order to design more effective regulators of this important biological process. During the course of preliminary kinetic studies on the transmethylation reaction catalyzed by catecholamine-O-methyltransferase (COMT, EC 2.1.1.6), a strong inhibition by one of the products, S-adenosylhomocysteine (SAH), was noted.† Product inhibition by SAH has been reported for enzyme-catalyzed Me transfer to such diverse acceptor molecules as homocysteine,[3] histamine,[4] N-acetylserotonin,[4] and transfer ribonucleic acid (tRNA).[5,6] The product inhibition of these various transmethylases by SAH presumably is regulated by the further breakdown of SAH to adenine and ribosylhomocysteine.[7] It has been shown recently that a macromolecular component of brain homogenate can activate COMT and phenethanolamine N-methyltransferase[8] (PNMT, EC 2.1.1.). This macromolecule has been shown to be an enzyme which can degrade SAH, and its stimulatory effect is thought to be due to the destruction of the natural inhibitor, SAH.

Most studies on inhibition of COMT[9] and PNMT[10] have utilized analogs of the acceptor substrate; *i.e.*, catechol for COMT, and phenethanolamine for PNMT. Kinetic studies† suggest that SAM and SAH bind more tightly to COMT than does either the acceptor substrate or the methylated product. In consideration of the data summarized above, we have synthesized analogs of SAH which do not contain the readily hydrolyzed glycosidic linkage. These compounds have been tested as inhibitors of COMT isolated from rat liver.[11] All contain adenine, a 5-C "bridge", and a thioether residue structurally related to homocysteine. The general structure of the compounds required in this study is shown in Figure 1, with SAH being shown for comparative purposes. In addition, several simple alkyl derivatives of homocysteine were synthesized.

Chemistry. In general, derivatives of homocysteine are prepared by reaction of S-benzylhomocysteine in Na-liq NH_3 to generate homocysteine which can then be condensed *in situ* with the requisite halide[12] or tosylate.[13] For synthesis of the open-chain analogs of SAH (Figure 1b), the procedure shown in Scheme I seemed appropriate. The precursor alcohol (**1**)[14] was prepared by the method of Santi and Peña‡ *via* direct alkylation of the Na salt of adenine[15] with 5-chloroamyl acetate, followed by basic hydrolysis of the resultant 9-(5-acetoxy)adenine. All attempts to prepare the tosylate ester of **1** (**2**), using a variety of solvents and reaction conditions, resulted in recovery of starting alcohol. Attention was then turned to preparation of the corresponding halide **3** or **4**. The synthesis of **3** had been reported previously[15] *via* direct alkylation of the Na salt of adenine with an excess of 5-bromo-1-chloropentane. A similar reaction using 1,5-dibromopentane failed to give **4** in our hands, so that **1** was converted to **3**·HCl by treatment with $SOCl_2$,[16] followed by neutralization to give the free base **3**. Condensation of **3** with S-benzylhomocysteine (**5**)[17] gave the desired SAH analog **6** after column chromatography on Dowex 1.

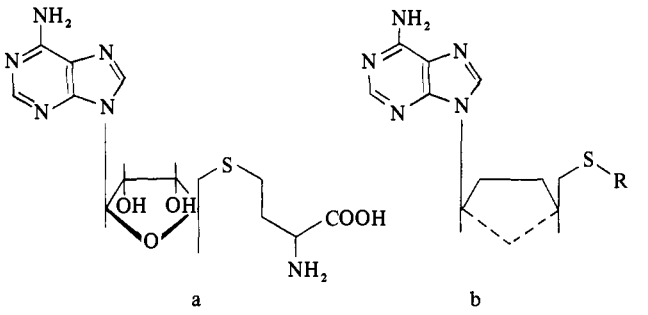
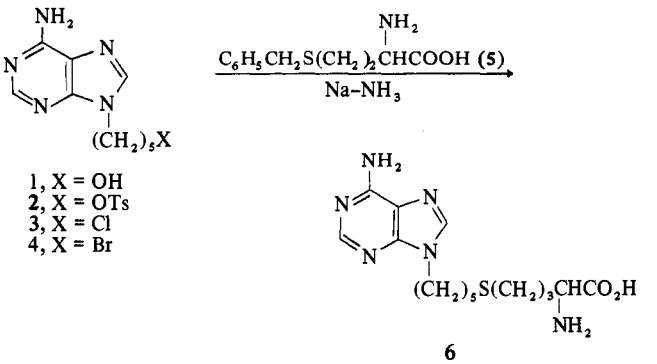


Figure 1. Structural formulas of (a) SAH, and (b) analogs synthesized in this study.

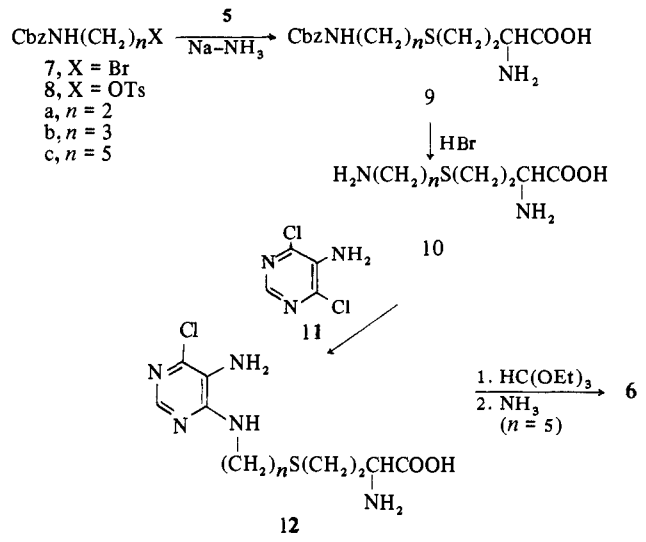
An alternate to the above route is to attach the homocysteine residue to the "bridge" prior to attaching the heterocyclic base. The synthetic route chosen is shown in Scheme II. The b series ($n = 3$) was selected for initial investigation due to the availability of starting materials. **7b** and **8b** were prepared exactly as described for **7a**[18] and **8a**.[19] The resulting oils, which had the expected spectral characteristics and were homogeneous by tlc, were suitable for use in subsequent transformations. Condensation of either **7b** or **8b** with **5** gave **9b**, which was readily deblocked with HBr-HOAc to give **10b**·HBr after work-up. Utilization of the normal procedures[20] for condensing amines with **11**[21] failed to produce any **12b**, presumably due to

Scheme I



†J. K. Coward and M. D'Urso-Scott, unpublished observations.
‡D. V. Santi and V. Peña, personal communication.

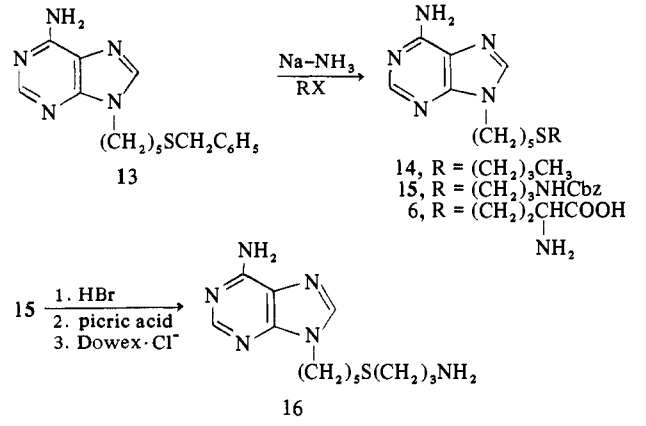
Scheme II



the inability to obtain the free base **10b**, even in the presence of strong organic bases in org solvents. Chromatographic data indicated that **10b** and **11** were recovered essentially unchanged. Condensation of **10b** and **11** was finally achieved in an aq methoxyethanol solvent system, using KOH to liberate the base **10b**, and Et_3N for neutralization. Good yields of **12b** were obtained by this procedure, and the amorphous product was homogeneous by tlc. The low solubility of **12b** in most organic solvents caused problems in the reaction with triethyl orthoformate, and no conditions were found that would effect cyclization of **12b** to the 6-chloropurine derivative.

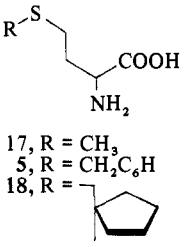
In order to synthesize a variety of compounds indicated in Figure 1b, a more general route was desirable. A convenient method would be to treat a simple alkyl residue containing desired functional groups with an adenine derivative to which the "bridge" and S atom was already attached. The route chosen for this work is shown in Scheme III. Benzylmercaptan and 5-bromovaleronitrile

Scheme III



were condensed and the product reduced with LAH in a manner similar to that reported for the *S*-Me compound.[22] The resulting amine was then condensed with **11** by the customary procedure,[21] followed by cyclization and amination to give the desired adenine derivative (**13**). Condensation of **13** with *n*-BuI or **7b** in the presence of Na-NH_3 gave the desired adducts **14** and **15**. The formation of **16** was readily accomplished by deblocking the alkylamine group of **15** with HBr, followed by conversion to the dihydrochloride *via* the dipicrate. Attempts to condense **13** with 4-chlorobutyric acid, 4-bromobutyronitrile, ethyl bromoacetate, or 4-bromo-2-aminobutyric acid[23] all failed to produce the desired adducts. Facile proton transfer, in addition to various intra- and intermolecular self-condensation reactions of C acids undoubtedly limit the use of alkyl halides containing acidic protons in this synthetic method.

Early results in this study demonstrated a drastic reduction in inhibitory activity by simply removing the homocysteine residue of SAH (*i.e.*, adenosine or adenine). In order to evaluate the contribution of the adenine-ribose portion of SAH to the binding energy, it was decided to study several derivatives of homocysteine as inhibitors of COMT-catalyzed transmethylation. The general structure shown below indicates the R groups selected as initial test compounds. Commercial methionine was used and **5** was available from the work discussed above, so that only **18** had to be synthesized to complete the series. This was accomplished by treating cyclopentylmethyl bromide[24] with **5** in Na-NH_3 as discussed previously.



Inhibition of COMT. The results of the inhibiton studies are shown in Table I. Unfortunately several of the compounds were too insoluble in aqueous media at 10^{-3}-10^{-4} *M* to be assayed as COMT inhibitors. None of the soluble acyclic analogs synthesized in this work have the potent inhibitory activity of SAH. The fact that adenine and adenosine are not good inhibitors of this reaction is not surprising in view of studies on tRNA methylase inhibition[25] where high concentrations of inhibitor are required. More closely related to the present work is a portion of an elegant study[3] on various derivatives of SAM as substrates and inhib-

Table I. Inhibition of Catechol-*O*-methyltransferase by Analogs of *S*-Adenosylhomocysteine[a]

Compd	Concn, m*M*	%, Inhibition[b]
SAH	0.42	78.1 ± 4.4
	0.62	88.6 ± 1.0
6	0.60	3.8 ± 1.9
	3.0	9.6 ± 2.3
	6.0	25.5 ± 11.8
16	5.4	8.9 ± 4.0
Adenine	0.60	20.0 ± 7.5
	6.0	26.7 ± 3.1
Adenosine	0.60	9.5 ± 1.9
	6.0	26.9 ± 1.1
MTA	0.54	22.0 ± 8.9
	5.4	32.3 ± 1.3
18	0.60	2.3[c]
Methionine	3.0	−13.0 ± 1.5
	6.0	−26.2 ± 0.6

[a] For assay condns, see Experimental Section. SAH = *S*-adenosylhomocysteine; MTA = 5′-methylthioadenosine. Solubility limitations prevented the assay of **5**, **14**, and **15** in the concn ranges employed above. In addition, it was not possible to assay **18** at >0.6 m*M* due to its low solubility in aqueous media. [b] % Inhibition data are representative of several experiments. All individual points were run in triplicate in each experiment. [c] Result of a single experiment.

itors of several transmethylases. In particular, it was observed that at concentrations equal to 10 times that of the substrate, 5′-methylthioadenosine (MTA), and decarboxylated SAM, showed only 19 and 29% inhibition, respectively. This is in agreement with the present work, using MTA and the analog of decarboxylated SAH, **16**. More surprising was the lack of potent inhibitory activity of **6**. Although this compound does inhibit the COMT reaction at high concentrations its activity is not markedly different than other 9-substituted adenines listed in Table I. The apparent activation of the reaction by methionine is not readily explainable at this time.

In contrast to studies with adenosine deaminase,[26] this work indicates that acyclic analogs of adenosine (SAH) bind only weakly as inhibitors of the enzymic reaction catalyzed by COMT. Presumably, analogs which are structurally more rigid than **6**, such as those containing a cyclopentyl moiety in place of ribose,[27] will be required to mimic the potent inhibitory activity of SAH.

Experimental Section§

9-(5-Chloropentyl)adenine (3). 9-(5-Hydroxypentyl)adenine (1) was prepd by alkylation of the Na salt of adenine with 5-chloroamyl acetate in DMF, followed by hydrolysis of the resulting AcO compd in methanolic NH_3;[15] mp 186.5–190° (lit.[14] mp 180–187°). The alcohol 1 (440 mg, 2 mmoles) was added to 10 ml of $SOCl_2$, and the resulting mixture heated under reflux for 30 min. Excess $SOCl_2$ was removed under reduced pressure and the resulting white residue crystd from EtOH to give 331 mg (56%) of 3 · HCl, mp 189–190°. Tlc (silica gel) in several solvent systems showed that the product was not identical with 1. *Anal.* ($C_{10}H_{14}ClN_5 \cdot HCl$) C, H, N. An aqueous soln of 3 · HCl was neutralized with Na_2CO_3 and the resulting ppt sepd by filtration to give 3 (92%); mp 154.5–156° (lit.[16] 155–157°).

9-[*S*-(4-(2-Amino)butyric acid)-5′-thiopentyl]adenine (6). *S*-Benzyl-DL-homocysteine (5)[17] (760 mg, 2.76 mmoles) was dissolved in 25 ml of liq NH_3 surrounded by a Dry Ice–acetone bath, and an excess of Na (*ca.* 130 mg, *ca.* 5 mg-atom) added in small portions until a deep blue color persisted for 15 min.[13] The chloride 3 (798 mg, 3.3 mmoles) was added to the blue soln in 1 portion, as the color was discharged within 5–10 sec. The resulting mixt was stirred for an addnl 10 min, the stirrer and bath were then removed, and NH_3 was allowed to evap slowly over a 3- to 4-hr period. The residue was partitioned between H_2O and Et_2O, and the aqueous layer was adjusted to pH 4.0 with HCOOH. This soln was evapd under reduced pressure at 40°, and finally lyophilized to give 1.5 g of crude product. Tlc on cellulose [*n*-BuOH–HOAc–H_2O (12:3:9)] indicated a mixt of products with 1 major spot (uv-absorbing and ninhydrin-positive) at R_f 0.76. A 200-mg portion of this crude product was dissolved in 8 ml of 0.1 *M* $HCOONH_4$, the pH adjusted to 10.5, and the resulting soln applied to a Dowex 1-X8 ($HCOO^-$ form) column (220 × 20 mm). The column was first washed with H_2O, and then the product eluted with a linear pH gradient of 0.1 *M* $HCOONH_4$ (pH 9.88 to pH 2.82). The effluent was monitored by uv absorption (256 nm). The product with R_f 0.76 was eluted with 580 to 715 ml of buffer, pH 6.5–4.8. The pooled fractions were lyophilized to give 63 mg (51%) of a white powder; mp 205–215° dec with softening at 190°, and no residue on burning; λ_{max} 260 nm. *Anal.* ($C_{14}H_{22}N_6O_2 \cdot 0.5HCOOH$) C, H, N.

S-(3-Aminopropyl)homocysteine (10b). *N*-(Carbobenzyloxy-3-amino-1-propyl tosylate (8b) was prepd from the corresponding alcohol[28] in a manner exactly analogous to that reported for 8a.[19] The oily tosylate, 8b (11.6 g, 31.8 mmoles), dissolved in 60 ml of liq NH_3, was added to a soln of Na_2 salt of homocysteine, derived from 5.63 g (25 mmoles) of **5** in 145 ml of liq NH_3 and 1.16 g (50 mg-atoms) of Na as described above for **6**. The residue remaining after evapn of NH_3 was dissolved in 90 ml of H_2O and 2.3 ml of 0.5 *M* EDTA. The pH was adjusted to 7.0 with 1 *N* H_2SO_4 as a white ppt formed. This was filtered off, and the solid product washed with H_2O, and then Me_2CO. The powder was dried to give 6.2 g (77%) of 9b; mp 216–225° dec, softening from 190°. This material was suitable for further transformations. Thus, 9b (6.1 g, 18.7 mmoles) was added to 25 ml of 40% HBr–HOAc, and the soln was stirred at ambient temp for 1 hr as CO_2 was given off. Et_2O (250 ml) was added in 50-ml portions and the resulting amorphous ppt was dissolved in a minimum of hot EtOH. The product was pptd by addn of pyridine to turbidity. The resulting cryst material was dried to give 4.05 g (78%) of 10b · HBr; mp 242–246°. Recrystn from H_2O–EtOH afforded an off-white cryst material; net wt 3.38 g (65%); mp 252–254° dec. *Anal.* ($C_7H_{16}N_2O_2S \cdot HBr$) C, H, N.

5-Amino-4-chloro-6-[3-(homocysteinyl)-*n*-propyl]aminopyrimidine (12b). A soln of 10b was prepd by dissolving 819 mg (3 mmoles) of 10b · HBr in 3 ml of H_2O, and then adding 7.0 ml (6 mequiv) of 0.856 *M* KOH to liberate the base. Et_3N (0.67 ml, 4.8 mmoles) was then added to the aqueous soln, followed by dropwise addn of a soln contg 738 mg (4.5 mmoles) of 11 in 8 ml of methoxyethanol. The resulting mixt was heated under reflux for 15 hr as a homogeneous, light yellow soln was observed. The reaction was conveniently monitored by tlc and uv. The soln was concd *in vacuo* to give an oily brown residue, which was triturated several times with hot ligroin to remove any unreacted 11. The resulting amorphous residue was homogeneous by tlc [cellulose, BuOH–HOAc–H_2O (10:3:9)] and was suitable for use in further transformations.

5-Benzylthiovaleronitrile. Benzylmercaptan (16.5 ml, 140 mmoles) was added slowly to a soln of 3.22 g (140 mmoles) of Na in 175 ml of MeOH. To this soln was added 20.25 ml (145 mmoles) of 5-bromovaleronitrile in 25 ml of MeOH and the resulting soln heated under reflux for 17 hr. The solvent was removed under reduced pressure, and the residue partitioned between H_2O and $CHCl_3$. The aqueous layer was washed with $CHCl_3$ and the combined dried organic layers were concd *in vacuo* to give an oily yellow residue. Distn of this residue afforded 23.49 g (82%) of product, bp 158–160° (0.85 mm). An analytical sample was obtd by redistg a small portion of the product; bp 143–144° (0.6 mm). *Anal.* ($C_{12}H_{15}NS$) C, H, N.

5-Benzylthiopentylamine. LAH (6.4 g, 169 mmoles) was suspended in 130 ml of Et_2O. A soln of 23.49 g (113 mmoles) of 5-benzylthiovaleronitrile in 70 ml of Et_2O was added dropwise to the LAH suspension over a period of 40 min to maintain a gentle reflux. The resulting mixt was stirred at ambient temp for 1 hr, then heated under reflux for an addnl 3 hr. Excess LAH was decompd by dropwise addn of H_2O (10 ml), then 2.5 *M* NaOH (10 ml), and finally H_2O again (25 ml). The product was extd from the mixt by several Et_2O and $CHCl_3$ washes. The combined org layers were dried, concd *in vacuo*, and distd to give 8.42 g (36%) of product; bp 115–116° (0.4 mm). *Anal.* ($C_{12}H_{17}NS$) C, H, N. Similar prepns afforded the product in 40–50% yield.

9-(*S*-Benzyl-5-thiopentyl)adenine (13). To a soln of 5-benzylthiopentylamine (2.30 g, 11 mmoles) and 1.65 ml (12 mmoles) of Et_3N in 35 ml of *n*-BuOH, was added 1.64 g (10 mmoles) of 4,6-dichloro-5-aminopyrimidine (11),[21] and the soln heated under reflux for 12 hr. The solvent was removed under reduced pressure and the residue extd with hot H_2O (2 × 25 ml). EtOH was added to effect transfer of the org material, and the soln concd *in vacuo* to give a quant yield of the substituted diaminopyrimidine, λ_{max} 295, 265 nm, which was used without further purification. A soln of 3.0 g (9 mmoles) of pyrimidine in 30 ml of $(EtO)_3CH$ was treated with 1 ml of 12 *N* HCl dropwise, and the resulting yellow soln was stirred at ambient temp for 20 hr. The reaction soln was then concd *in vacuo* to give a brown oil; net wt 2.6 g (85%), λ_{max} 265 nm. This material was dissolved in 25 ml of MeOH, and the resulting soln treated with charcoal. The decolorized soln was placed in a Parr stainless steel bomb and satd with NH_3 at 0°. The bomb was placed in an oil bath at 85° for 15 hr, after which time the mixt was cooled and the solvent removed *in vacuo*. The semisolid residue was extd with $CHCl_3$, and the $CHCl_3$ exts were concd *in vacuo* to give 1.07 g of 13, a beige cryst material (43%, 33% overall from 5-benzylthiopentylamine); mp 130–134°. Several recrystns from aqueous EtOH gave a white crystalline analytical sample, mp 130.5–133.5°. *Anal.* ($C_{17}H_{21}N_5S$) C, H, N, S.

9-(*S*-Butyl-5-thiopentyl)adenine (14). A soln of 332 mg (1 mmole) of 13 in 10 ml of liq NH_3 was treated with *ca.* 40 mg (1.7 mg-atoms) of Na in small portions until a deep blue color persisted

§ All melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements or functions were within ±0.4% of the theoretical value. Ir spectra were run on a Perkin-Elmer Model 21 spectrophotometer, and uv spectra on a Cary Model 15 spectrophotometer. Tlc were run on Eastman chromatograms #6060 (silica gel with fluorescent indicator), or #6065 (cellulose with fluorescent indicator). Spots were detected by visual examination under uv light and/or with ninhydrin, or PtI_4 spray reagents, for compds contg amino or thioether moieties, respectively.

for 15 min. Then 232 mg (1.26 mmoles) of *n*-BuI was added in 1 portion and the blue color was immediately discharged. The resulting soln was stirred in a Dry Ice–acetone bath for 30 min, then was removed from the bath and stirred as the NH_3 was allowed to evap slowly overnight. The residue was dissolved in 10 ml of H_2O, the soln adjusted to pH 8.0 with HCl, and then extd with $CHCl_3$ (3 × 10 ml). The dried $CHCl_3$ exts were concd *in vacuo* and the residual oil was triturated with petr ether (30–60°) to give a solid material. Crystn from EtOH gave 95 mg (32%) of yellow crystals; mp 87–88.5°, λ_{max} 260 nm. *Anal.* ($C_{14}H_{23}N_5S$) C, H, N.

9-[*S*-(3-Aminopropyl)-5-thiopentyl]adenine · 2HCl (16). The reaction between 13 (221 mg, 1 mmole) and 7b (342 mg, 1.26 mmoles) was carried out with Na–NH_3 exactly as described for the prepn of 14. The oily residue (15) thus obtained was treated with 40% HBr–HOAc overnight at ambient temp to cleave the carbobenzyloxy blocking group. The reaction soln was poured into 50 ml of Et_2O to give a very hygroscopic HBr salt, which was dissolved in EtOH, and treated with charcoal. The decolorized filtrate was treated with an equal vol of a satd soln of picric acid in EtOH, to ppt 194 mg of a yellow solid. Recrystn from EtOH gave an analytical sample; mp 176–180° dec with preliminary softening. *Anal.* ($C_{25}H_{28}N_{13}O_{14}S$) C, H, N. The dipicrate could be converted to the dihydrochloride by chromatog of an EtOH soln on Dowex (X-8, Cl^- form), followed by concn of the effluent *in vacuo*; λ_{max} 260 nm; mp 174° dec.

S-(Cyclopentylmethyl)homocysteine (18). To a deep-blue soln derived from 2.05 g (9.1 mmoles) of 5 and 400 mg (17.4 mg-atoms) of Na in 35 ml of liq NH_3, was added 2.05 g (12.5 mmoles) of cyclopentylmethyl bromide.[24] The blue color disappeared immediately, and the mixt was worked up as described for 9b. The dried white solid weighed 2.22 g, and was crystd from 95% EtOH to give an analytical sample; mp 187–188° dec, with preliminary darkening at 165°. *Anal.* ($C_{10}H_{20}NO_2S$) C, H, N.

Enzyme Assay. The assay method of Nikodejevic, *et al.*,[11] was used with minor modifications; namely, the concn of SAM was 6 × 10^{-4} *M* (contg 10^{-2} μCi of [*methyl*-^{14}C]SAM), and pH was maintd at 7.9 with 0.1 *M* Tris buffer. The specific activity of the enzyme prepns employed ranged from 2.8 × 10^{-3} to 5.6 × 10^{-2} μmole of product/mg of protein per min. Protein concn was detd by uv absorbance at 280 and 260 nm.[29]

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Conformation of Gastrin Tetrapeptide

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The conformation of the amino acid residues comprising gastrin tetrapeptide have been predicted using semiempirical molecular orbital theory. The conformation of gastrin tetrapeptide has been predicted by a direct assembly of these residues in their preferred conformations. The implication of this predicted structure is discussed in terms of the biological activity.

Gastrin is a heptadecapeptide, released by the gastric mucosa, stimulating gastric acid secretion, gastric motility, and pancreatic secretion.[1-3] Studies to date support the contention that gastrin does not act at sites receptive to histamine.[1] There appears to be a strong interdependence between gastrin and acetylcholine; the hypothesis that gastrin stimulates the release of ACh[4] receives considerable support.[5]

The amino acid sequence of gastrin has been defined as two natural forms of a heptadecapeptide: gastrin-II, with a sulfated tyrosyl residue, and gastrin-I, unsulfated.[6] Tracy and Gregory discovered that the entire range of physiological activity of gastrin could be demonstrated by the C-terminal tetrapeptide sequence Trp-Met-Asp-Phe·NH_2.[7] This intriguing observation prompted Morley to synthesize a large number of analogs of this tetrapeptide in an effort