in EtOH (10 mL). This was subjected to hydrogenation at 40 psi for 3 h. The suspension was filtered through Celite and washed with EtOH (50 mL). Evaporation in vacuo gave an oil, which was dissolved in hot EtOH (10 mL). Et_2O was added to the cloud point and the mixture cooled at 5 °C overnight. Filtration gave **5f** (717 mg, 75% yield), mp 124–125 °C dec. Anal. ($C_{12}H_{18}BrNO_2$·HCl) C, H, N.

3-(2′-Bromoethyl)-4-methoxyphenethylamine Hydrochloride (5g). This was prepared by the same procedures as for **12**, from **15** oxalate (299 mg, 1 mmol) and PBr_3 (0.22 mL, 2.3 mmol). The crude free base was dissolved in EtOH (15 mL), and 12 M HCl (0.10 mL) was added. Evaporation gave an oil, which was coevaporated with EtOH (2 × 10 mL) to give a white solid. This was dissolved in hot EtOH (10 mL), and Et_2O (5 mL) was added slowly. After cooling to 5 °C overnight, the crystals were filtered and washed with Et_2O to give **5g** (133 mg, 40%), mp 131–132 °C. Anal. ($C_{12}H_{18}BrNO$·HCl) H, N; C: calcd, 46.70; found, 47.17.

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

Method B. To EtOH (20 mL) and H_2O (10 mL) was added **5e** (1.24 g, 3 mmol) and thiourea (456 mg, 6.0 mmol). After the mixture was refluxed overnight, 2 N NaOH (6 mL) was added and refluxing continued for 15 min. The mixture was poured into H_2O (20 mL) and extracted with $CHCl_3$ (3 × 30 mL), dried ($MgSO_3$), filtered, and evaporated in vacuo to give a clear oil. This oil was dissolved in EtOH (30 mL) and 12 N HCl (0.30 mL) was added. Evaporation gave an oily solid, which was triturated in Et_2O to afford **6e** (900 mg, 90%) as a white solid. Crystallization from hot EtOH–Et_2O gave an analytical sample of **6e**, mp 148–149 °C (Table II).

Homocysteines (19). Method C. To 30 mL of MeOH in which Na (266 mg, 11.6 mmol) was previously dissolved was added **17** (918 mg, 6.0 mmol). After the mixture was stirred for 15 min, **5d** (1.075 g, 5.0 mmol) was added, and the reaction was stirred for 1.5 h. Evaporation gave an oily white solid, which was triturated with Et_2O (100 mL) and filtered. Evaporation of the filtrate gave the methyl ester as an oil (900 mg). This oil was dissolved in MeOH (10 mL), 1 N NaOH (10 mL) was added, and the mixture was stirred for 1 h. Evaporation to 10 mL, followed by neutralization to pH 7 with HCl, gave a white precipitate. Filtration afforded **19d** (670 mg, 50%), mp 192–195 °C dec (Table III).

5′-Thioadenosines (24). Method D. To a solution of 400 mg (10 mmol) of NaOH in 15 mL of H_2O was added 1.45 g (5 mmol) of the isothiouronium salt **6d**, and the resulting mixture was heated at 80 °C for 1 h under N_2, at which time 570 mg (2.0 mmol) of **23** was added, and heating continued under N_2 for an additional 1 h. The reaction mixture was then cooled, the solution was adjusted to pH 6 with glacial HOAc, and the aqueous supernatant was decanted. The residue was triturated with Et_2O to give a white solid, which was then crystallized from EtOH–Et_2O, followed by recrystallization from EtOH–H_2O, to yield 610 mg (77%) of **24d**. An analytical sample was obtained after two recrystallizations from CH_3OH, mp 104–105 °C (Table IV).

Method E. To a solution (5 mL) of 2 N NaOH previously purged with N_2 was added 285 mg (1 mmol) of **23** and 368 mg (1 mmol) of **6e**. After heating under N_2 at 70 °C for 4 h, the reaction mixture was cooled and extracted with EtOAc (6 × 5 mL), and the dried organic extract was concentrated in vacuo. The resulting residue was dissolved in MeOH, and the desired product, **24e**, slowly precipitated from solution: yield 250 mg (43%) of a white solid. Recrystallization from CH_3OH gave an analytical sample, mp 145–147 °C (Table IV).

Sulfonium Salts (3 and 4). Method F. The appropriate thioether (1 mmol) was dissolved in formic acid (2.5 mL) and stirred in the dark with MeI (0.30 mL, 5 mmol) until the reaction was judged to be complete by ^1H NMR (3–72 h). The mixture was poured in ice (10 g) and extracted with Et_2O (3 × 10 mL). The aqueous layer was lyophilized to give the iodide salt of the sulfonium compound, which was dissolved in H_2O and passed through an anion resin column in ClO_4 form. The aqueous eluent was lyophilized to give the sulfonium perchlorate as a white powder (Tables III and IV).

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

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

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

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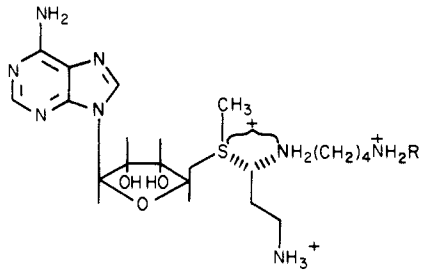
(1) H. G. Williams-Ashman and A. E. Pegg, in "Polyamines in Biology and Medicine", D. R. Morris and L. Marton, Eds., Marcel Dekker, New York, in press.

0022-2623/81/1824-1277$01.25/0

echol *O*-methyltransferase (COMT), proceeds by a random, sequential kinetic mechanism[2] via direct nucleophilic attack of the catechol hydroxyl group on the methyl carbon of SAM,[3] probably involving general-base-catalyzed proton abstraction.[4] Our earlier studies demonstrated a nonspecific inhibition of SAM-dependent methylases and aminopropyltransferases by the nucleoside products *S*-adenosylhomocysteine (SAH) and 5′-(methylthio)-adenosine (MTA), respectively.[5,6] Therefore, we have initiated a research program aimed at improving the specificity of synthetic inhibitors of alkyl transfer reactions by incorporating in the inhibitor molecules structural features of proposed enzyme-bound transition states. Reviews by Wolfenden[7] and Jencks[8] give detailed discussions of the basis for the hypothesis that "transition-state analogues" should be extremely potent inhibitors of enzyme-catalyzed reactions, an hypothesis which is now supported by a considerable body of experimental evidence.[7]

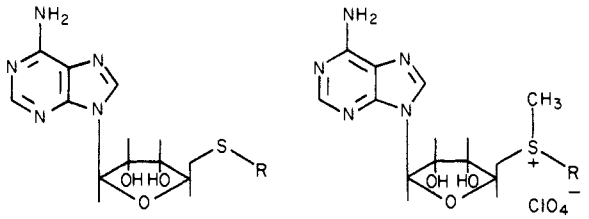
The direct displacement mechanism indicated for the COMT reaction[3] suggested to us that the APT reactions might proceed via a similar direct displacement, as shown in **1**. This mechanism indicates that nucleophilic attack



1

a, R = H
b, R = $(CH_2)_3NH_3^+$

on dcSAM by putrescine, catalyzed by spermidine synthase, involves transition state **1a**, whereas nucleophilic attack on dcSAM by spermidine, catalyzed by spermine synthase, involves transition state **1b**. In this paper we describe the syntheses of **2b** and **3b**, potential transition-

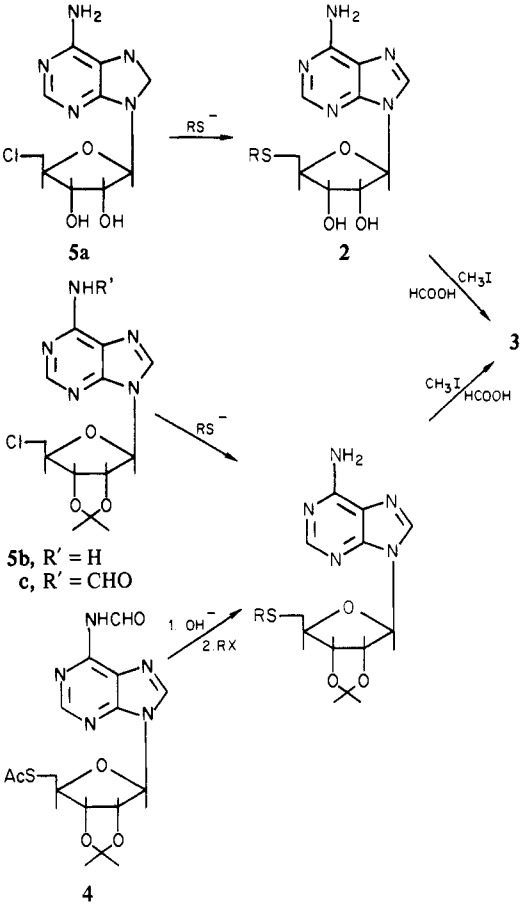


2 **3**

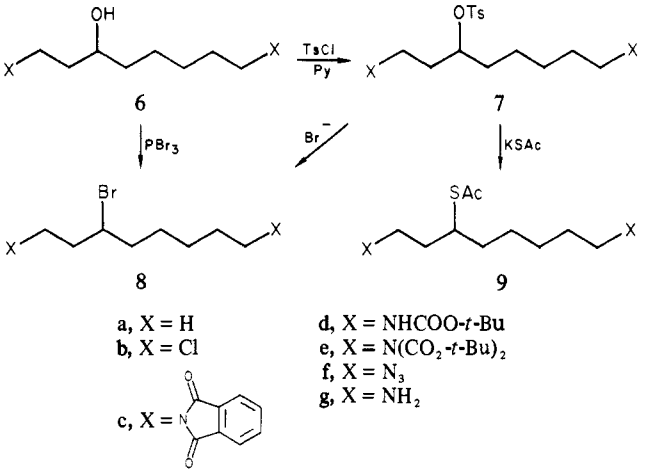
a, R = CH_3
b, R = $CH(CH_2CH_3)(CH_2)_4CH_3$
c, R = $CH[(CH_2)_2NH_3^+](CH_2)_5NH_3^+$
d, R = $CH[(CH_2)_2N_3](CH_2)_5N_3$

state analogue inhibitors of spermidine synthase. The

Scheme I



Scheme II



a, X = H
b, X = Cl
c, X =
d, X = NHCOO-*t*-Bu
e, X = $N(CO_2$-*t*-$Bu)_2$
f, X = N_3
g, X = NH_2

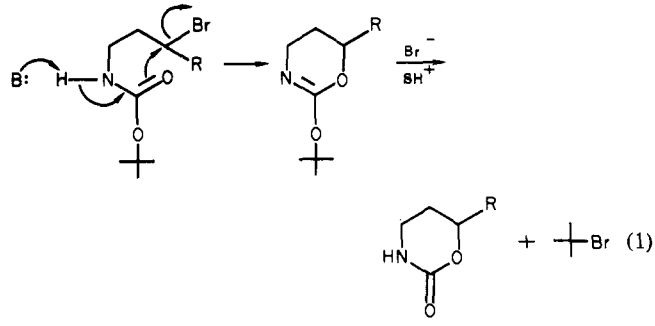
syntheses of the deamino analogues **2a** and **3a**, predicted to be very poor inhibitors of this enzyme based on substrate structure–activity data,[9] are also described. Preliminary kinetic studies on purified spermidine synthase from rat prostrate reveal that **2b** and **3b** are potent and specific inhibitors of this enzyme.[10,11]

(2) J. K. Coward, E. P. Slisz, and F. Y.-H. Wu, *Biochemistry*, 12, 2291 (1973).
(3) R. W. Woodard, M.-D. Tsai, H. G. Floss, P. A. Crooks, and J. K. Coward, *J. Biol. Chem.*, **255**, 9124 (1980).
(4) J. O. Knipe and J. K. Coward, *J. Am. Chem. Soc.*, **101**, 4339 (1979).
(5) J. K. Coward, D. L. Bussolotti, and C.-D. Chang, *J. Med. Chem.*, 17, 1286 (1974).
(6) H. Hibasami, R. T. Borchardt, S. Y. Chen, J. K. Coward, and A. E. Pegg, *Biochem. J.*, 187, 419 (1980).
(7) R. Wolfenden, *Annu. Rev. Biophys. Bioeng.*, 5, 271 (1976).
(8) W. P. Jencks, *Adv. Enzymol.*, 43, 219 (1975).
(9) K. Samejima and Y. Nakazawa, *Arch. Biochem. Biophys.*, **201**, 241 (1980).
(10) K.-C. Tang, A. E. Pegg, and J. K. Coward, *Biochem. Biophys. Res. Commun.*, **96**, 1371 (1980).
(11) After this work had been completed, a paper appeared[12] in which steady-state kinetic studies suggested a double-displacement ("ping-pong") mechanism for spermidine synthase isolated from *E. coli*. However, product inhibition studies were not done nor have these conclusions been confirmed by stereochemical investigations.

Chemistry. The general synthetic approaches to molecules of type **2** and **3** are shown in Scheme I. These routes are well-documented in the literature, including previous work from this laboratory.[13,14] Therefore, we investigated synthetic routes to the appropriate RX and RSH for ultimate coupling to 5′-deoxy-5′-thio- and 5′-deoxy-5′-chloroadenosine derivatives, respectively. A generalized scheme for the syntheses of the desired alkyl halides and alkyl thioacetates is shown in Scheme II. For the syntheses of **2b** and **3b** precursors, conversion of 3-octanone to the corresponding secondary alcohol **6a**, followed by reaction of the alcohol with PBr_3, led to 3-bromooctane (**8a**). The bromide **8a** was then converted directly to the desired thiol precursor **9a** via bromide displacement with KSAc in dimethyl sulfoxide. Coupling of **9a** to 5′-deoxy-5′-chloroadenosine (**5a**) led to the desired thioether **2b**, which could be methylated to **3b** with CH_3I in HCOOH. Similarly, **8a** could be coupled in low yield to the 5′-deoxy-5′-thioadenosine derivative, generated in situ from **4**, followed by methylation with CH_3I/HCOOH to give **3b**.

Our initial attempts on the syntheses of the diamino analogues **2c** and **3c** involved the preparation of the key intermediate 1,8-diphthalimido-3-bromooctane (**8c**) and the corresponding thioacetate **9c**. ϵ-Caprolactone was converted to 6-chlorohexanoyl chloride via $ZnCl_2$-catalyzed lactone ring opening in the presence of $SOCl_2$. Ethylene addition to the acid chloride, catalyzed by $AlCl_3$, led to the unstable β-chloro ketone, 1,8-dichloro-3-octanone, which could be reduced with $NaBH_4$ to give 1,8-dichloro-3-octanol (**6b**). Displacement of the chloride atoms of **6b** by potassium phthalimide in DMF gave **6c**, which could be converted to the desired 3-bromo derivative **8c** via the corresponding tosylate **7c**. Conversion of the bromide **8c** to the thioacetate **9c** was again effected by reaction of **8c** with KSAc in Me_2SO. Unfortunately, neither **8c** nor **9c** could be successfully coupled to the appropriate adenosine derivatives (Scheme I). The in situ generation of 5′-deoxy-5′-thioadenosine from **4**, followed by addition of the secondary bromides **8**, led to large amounts of the adenosine disulfide,[13,14] which, in the case of the least complex bromide, **8a**, led to low yields of the desired 5′-thioether, even when the reaction was run in the absence of oxygen. In the case of the attempted coupling of **8c**, an additional problem was encountered, namely, apparent attack of the thiolate anion on the phthalimide carbonyl groups. Similarly, attempted coupling of the thiolate generated in situ from **9c** led to partial cleavage of the phthalimide group under the basic reaction conditions.

The use of the *tert*-butoxycarbonyl (Boc) group to protect the amine functions offered the advantage of base stability, which presumably would avoid the problems encountered with the phthalimide derivatives just described. The introduction of the Boc function is generally accomplished by derivatization of the free amine, which was not appropriate for our work. However, the recent description of the imidies $HN(Boc)(CO_2CH_3)$ and $HN(Boc)_2$[15] led us to investigate the possible use of the Boc group in these syntheses. Conversion of 1,8-dichloro-3-octanol to either the 1,8-$[N(Boc)(CO_2CH_3)]_2$ or 1,8-$[N(Boc)_2]_2$ derivatives was effected by reaction of the intermediate 1,8-diiodo-3-octanol with an alkali salt of the appropriate imide in DMF. Base hydrolysis of the methyl ester function in the 1,8-$[N(Boc)(CO_2CH_3)]_2$ derivative led to the unstable carbamic acid, which spontaneously decarboxylated to give **6d**. This material could be converted to 1,8-diamino-3-octanol (**6g**) by treatment with CF_3CO-OH. However, all attempts to obtain either the tosylate **7d** or bromide **8d** failed, presumably due to facile intramolecular cyclization (eq 1), as previously demonstrated



(1)

with other carbamates under neutral conditions.[16] The loss of ~50% of the *tert*-butyl in the recovered product is consistent with the reaction shown in eq 1. The *N*,*N*,*N*′,*N*′-tetrakis(*tert*-butoxycarbonyl)-3-hydroxy-1,8-octanediamine (**6e**) lacking the free NH of the Boc derivative **6d** should lead to a more stable tosylate (**7e**) or bromide (**7f**). Although it proved impossible to obtain **6e** as a distillable or crystalline product, a chromatographically and spectrally pure oil was obtained and was converted to the bromide **8e** via the tosylate (**7e**). Both of these materials were viscous oils which were pure by spectral and chromatographic criteria but which defied all crystallization attempts. Coupling of **8e** to the 5′-thioadenosine derivative, generated in situ from **4**, led to the totally blocked adenosine thioether precursor of **3c**, isolated as an amorphous solid. Methylation of this material with CH_3I in HCOOH led to the totally deblocked sulfonium salt **3c**.

Although this route to **3c** was effective in obtaining the target compound, the inability to obtain distillable or crystalline synthetic intermediates in the conversion of **6b** to **8e** led us to seek an alternative synthetic strategy. The azide function appeared to be a plausible amine precursor for this work, and conversion of 1,8-dichloro-3-octanol (**6b**) to the corresponding 1,8-diazido derivative **6f** was accomplished using NaN_3 in DMF, containing catalytic amounts of LiI. Tosylation of the alcohol gave **7f**, which could be converted to the 1,8-diazido-3-(acetylthio)octane (**9f**). Coupling of the thiol, generated in situ by base hydrolysis of **9f**, to 5′-deoxy-5′-chloroadenosine (**5a**) gave the diazidooctylthioadenosine precursor **2d**. Reduction of the azide functions with triphenylphosphine/pyridine gave the desired diamino thioether **2c**. Surprisingly, attempted methylation of **2c** to give **3c** failed to yield appreciable amounts of the diaminosulfonium salt. Unreacted **2c** was the major recovered product, and it seemed possible that in HCOOH the positively charged amino functions of **2c** might lead to electrostatic repulsion(s) in the transition state leading to **3c**. Treatment of **2c** with $(Boc)_2O$ gave the 1,8-$(Boc)_2$ derivative which, on treatment with

(12) V. Zappia, G. Cacciapuoti, G. Pontoni, and A. Oliva, *J. Biol. Chem.*, **255**, 7276 (1980).
(13) J. K. Coward, N. C. Motola, and J. D. Moyer, *J. Med. Chem.*, **20**, 500 (1977).
(14) G. L. Anderson, D. L. Bussolotti, and J. K. Coward, *J. Med. Chem.*, preceding paper this issue.
(15) C. T. Clarke, J. D. Elliott, and J. H. Jones, *J. Chem. Soc., Perkin Trans. 1*, 1088 (1978).
(16) J. K. Coward and R. Lok, *J. Org. Chem.*, **38**, 2546 (1973).
(17) (a) J. Baddiley and G. A. Jamieson, *J. Chem. Soc.*, 1085 (1955). (b) T. Nielson, T.-Y. Shen, and W. V. Rayla, French Patent 589-694; *Chem. Abstr.*, **74**, p126013c (1971).
(18) J. Cason and J. S. Correia, *J. Org. Chem.*, **26**, 3645 (1961).

Table I. Inhibition of Spermidine Synthase and Spermine Synthase by 2 and 3 [a]

no.	concn, μM	spermidine synthase	spermine synthase
2b	25	102	89
	100	104	78
2c	10	3	
	25	3	90
	50	1	
	100	1	85
3b	10	98	94
	25	99	88
	50	93	65
	100	80	44
	250	56	25
3c	5	84	
	10	68	93
	25	31	
	50	16	60
	100	8	41
	250	4	14

[a] Enzyme inhibition data presented as percent of drug-free control.

CH_3I/HCOOH, rapidly lost both Boc groups but resisted methylation of the sulfur atom. The only explanation remaining would seem to involve the less rapid hydrolysis of the $N(Boc)_2$ group vs. the NHBoc group, thus allowing methylation of the neutral thioether derived from **9e** to occur prior to hydrolysis of the Boc groups. Thus, synthesis of **3c** would appear to require use of the $N(Boc)_2$ protecting group in the intermediates.

Biochemical Results

As noted several years ago in this laboratory, severe substrate inhibition by dcSAM is observed with spermidine synthase.[13] This makes the kinetic analysis of inhibition studies with this enzyme somewhat more complicated than in a normal system. We are currently carrying out more extensive kinetic studies on spermidine synthase. However, preliminary kinetic data (Table I)[10] reveal that **3c** is a reasonably potent inhibitor ($I_{50} \approx 15$ μM) of spermidine synthase and a much less effective inhibitor ($I_{50} \approx 80$ μM) of spermine synthase. The deaminosulfonium salt **3b** is a poor inhibitor ($I_{50} \approx 250$ μM) of spermidine synthase but comparable to **3c** as an inhibitor of spermine synthase and similar to other sulfoniums, such as **3a** and SAM, against the latter enzyme. Most encouraging, however, for the general approach being investigated are the data with **2c**, which is an extremely potent inhibitor ($I_{50} \approx 0.4$ μM) of spermidine synthase, while exhibiting almost no inhibitory activity against spermine synthase. Further kinetic studies are being pursued to investigate the nature of the enzyme–inhibitor interaction more completely. However, these preliminary data suggest that transition-state analogue inhibitors such as **2c** should be very useful for studying enzyme-catalyzed alkyl transfer reactions, notably aminopropyltransferases.

Experimental Section

All chemicals were of reagent quality and used without further purification with the following exceptions: pyridine and *N,N*-dimethylformamide (DMF) were dried over potassium hydroxide pellets and distilled, 2-butanone was distilled over $CaSO_4$ prior to use, dimethyl sulfoxide and hexamethylphosphoric triamide (HMPA) were distilled over calcium hydride prior to use, commercial tosyl chloride was recrystallized from petroleum ether–benzene, thionyl chloride was freshly distilled prior to use, and methanol was kept over molecular sieves (4Å). Potassium thioacetate was triturated with dry 2-butanone several times and dried in vacuo. Melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian T-60 spectrophotometer, IR spectra were measured using a Perkin-Elmer 237-B spectrophotometer, and UV spectra were measured using a Cary-15 or Perkin-Elmer 552 spectrophotometer. Elemental analyses were performed by Baron Consulting Co., Orange, CT. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of theoretical values. Thin-layer chromatography (TLC) was performed using either silica gel F-254 (EM Reagents) plates or Eastman cellulose plates with florescent indicator. Silica gel plates with florescent indicator for preparative chromatography were from Analtech (silica gel GF). Solvent systems used for TLC were as follows: (a) 1-BuOH/HOAc/H_2O, 12:3:5 (BAW); (b) 5% aqueous Na_2HPO_4; and (c) $CHCl_3$/MeOH, 4:1. High-performance liquid chromatography (HPLC) was performed using a Whatman ODS-2 reverse-phase column. 5′-Deoxy-5′-chloroadenosine (**5a**) was prepared by a modification of the literature procedure.[13,14] All compounds had spectral properties (NMR, IR, and UV) consistent with their assigned structures. All adenosylsulfonium compounds (**3**) (λ_{max} 259 nm) decomposed rapidly in 0.1 N NaOH to give adenine (λ_{max} 268 nm).[19]

5′-Deoxy-5′-chloro-2′,3′-isopropylideneadenosine (5b). Thionyl chloride (19.3 g, 19.5 mL, 162 mmol) was added dropwise to 150 mL of ice-cold dry hexamethylphosphoric triamide (HMPA) under a nitrogen atmosphere. To the resulting cooled solution was added 18.6 g (60 mmol) of 2′,3′-isopropylideneadenosine in portions. When the adenosine was added the color of the solution changed immediately from pale yellow to orange and then reddish. After 5 h of stirring at ambient temperature, the reaction mixture was poured into a well-stirred mixture of ice–water (900 g), and the reaction flask was rinsed with water. The aqueous solution thus obtained was adjusted to pH 9 with concentrated NH_4OH to give a white precipitate. The precipitate was collected and redissolved in chloroform. The chloroform solution was decolorized with charcoal to give a pale yellow solution, which was then poured into petroleum ether to give 17.2 g (88%) of a white precipitate with mp 278 °C dec. This material was shown to be homogeneous on TLC (silica; EtOAc/$CHCl_3$, 9:1) and HPLC (50% aqueous methanol, ODS-1): NMR ($CDCl_3$) δ 1.4 (3 H, s), 1.6 (3 H, s), 3.7 (2 H, dd, $H_{5'}$, J = 6 and 5 Hz), 4.46 (1 H, dt, $H_{4'}$, J = 2 Hz), 5.11 (1 H, dd, $H_{3'}$, J = 2 and 7 Hz), 5.48 (1 H, dd, $H_{2'}$, J = 2 and 7 Hz), 6.1 (1 H, d, $H_{1'}$, J = 2 Hz), 6.6 (2 H, br s, NH_2), 7.86 (1 H, s, H_2), 8.25 (1 H, s, H_8). Anal. ($C_{13}H_{16}ClN_5O_3$) C, H, N.

5′-Deoxy-5′-chloro-N^6-formyl-2′,3′-isopropylideneadenosine (5c). **5b** (2.22 g, 6.8 mmol) was dissolved in 20 mL of acetic–formic anhydride, and 0.8 g (7.55 mmol) of anhydrous sodium carbonate was added. The resulting solution was allowed to stir at ambient temperature for 8 h, and the reaction was monitored by TLC (silica gel; EtOAc/$CHCl_3$, 9:1). At the beginning, CO_2 evolution occurred and a slightly cloudy solution was obtained, but this became clear at the end of the reaction. The mixture was then concentrated to almost dryness under reduced pressure, and the residue was dissolved in 50 mL of $CHCl_3$, which was then washed with H_2O (2 × 40 mL), saturated aqueous $NaHCO_3$ (2 × 40 mL), and H_2O (40 mL) and dried over $MgSO_4$. After the solvent was removed 2.1 g (85%) of pure **5c** was obtained: mp 230 °C dec; NMR (Me_2SO-d_6) δ 1.28 (3 H, s), 1.49 (3 H, s), 3.82 (2 H, d, $H_{5'}$, J = 6 Hz), 4 41 (1 H, dt, $H_{4'}$, J = 2 and 6 Hz), 5.11 (1 H, dd, $H_{3'}$, J = 2 and 7 Hz), 5.54 (1 H, dd, $H_{2'}$, J = 7 Hz), 6.41 (1 H, d, $H_{1'}$, J = 2 Hz), 8.64 (1 H, s, H_2), 8.67 (1 H, s, H_8), 9.87 [1 H, d, –NHC(=O)–, J = 9 Hz], 11.24 (1 H, d, –CHO, J = 9 Hz). Anal. ($C_{14}H_{16}ClN_5O_4$) C, H, N.

5′-Deoxy-5′-(thioacetyl)-N^6-formyl-2′,3′-isopropylideneadenosine (4). **5c** (0.5 g, 1.42 mmol), previously triturated potassium thioacetate (0.485 g, 4.26 mmol), and a catalytic amount of anhydrous LiI were dissolved in 35 mL of 2-butanone, and the resulting solution was refluxed for 5 h. After cooling, the dark brown solution was filtered with the aid of Celite, and the filtrate was concentrated under reduced pressure with the bath temperature below 30 °C. The residue thus obtained was taken into 40 mL of chloroform and washed with H_2O (4 × 40 mL) and dried

(19) R. T. Borchardt, *J. Am. Chem. Soc.*, **101**, 458 (1979), and references therein.

over $MgSO_4$. After the solvent was removed, 280 mg of crude product was obtained, which, after recrystallization from $CHCl_3$–Et_2O, gave 235 mg (42.1%) of pure **4**, which exhibited spectral properties and melting point identical with that of an authentic compound prepared by the method published previously.[17]

3-Octanol (6a). To a well-stirred solution of 19.2 g (0.15 mmol) of 3-octanone in 270 mL of 95% ethanol, cooled in an ice bath, was added in portions a solution of 3.9 g (0.103 mmol) of sodium borohydride in 27 mL of water. Ammonium hydroxide (15 M, 27 mL) was added, the ice bath was removed, and stirring was continued at room temperature for 3 h. The reaction mixture was concentrated to near dryness, and the residue was partitioned between 250 mL each of $CHCl_3$ and H_2O. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (2 × 200 mL). The combined extracts were washed with 5% HCl (350 mL) and saturated NaCl solution (350 mL) and dried over $MgSO_4$. After the solvent was removed, the liquid residue was distilled under reduced pressure [bp 86–87 °C (24 torr), lit.[18] bp 69.5–70.4 °C (7 torr)] to give 15.41 g (79.02%) of pure **6a**: NMR ($CDCl_3$) δ 0.93 (6 H, t, CH_3), 1.37 (10 H, br m, CH_2), 2.35 (1 H, br s, exchangeable with D_2O and shift to low field in pyridine, OH), 3.47 (1 H, m, >CHO–).

3-Bromoctane (8a). To 8 mL (23 g, 85.3 mmol) of phosphorus tribromide was added dropwise 15 g (115.4 mmol) of 3-octanol (**6a**) over a 0.5-h period. The resulting solution was heated at 100 °C (oil bath) for 2 h, after which time the reaction mixture was cooled and poured into 200 mL of ice–water, which was extracted with $CHCl_3$ (3 × 120 mL). The combined organic extracts were washed with 5% $Na_2S_2O_3$ (2 × 200 mL), H_2O (200 mL), saturated aqueous $NaHCO_3$ (2 × 200 mL), H_2O (200 mL), and saturated aqueous NaCl (200 mL) and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the residue was distilled [bp 78–80 °C (18 torr), lit.[18] bp 84.4–85.1 °C (20 torr)] to give 17.5 g (78.58%) of pure **8a**, which exhibited a C–Br band at 797 cm^{-1} in the IR spectrum:[18] NMR ($CDCl_3$) δ 1.0 (6 H, t, CH_3), 1.31 (6 H, br s, CH_2), 1.75 [4 H, q, $CH_2C(Br)CH_2$], 3.90 (1 H, m, >CHBr).

***S*-Adenosyl-3-(methylthio)octane (3b). Route A. From 5′-Deoxy-5′-chloroadenosine (5a). 3-(Thioacetyl)octane (9a).** To 6.2 g (54.3 mmol) of previously triturated potassium thioacetate in 60 mL of dry Me_2SO was added 7.0 g (36.27 mmol) of 3-bromoctane (**8a**), and the resulting solution was stirred overnight at ambient temperature. The reaction mixture was then poured into 500 mL of H_2O and extracted with chloroform (2 × 250 mL). The combined $CHCl_3$ extracts were washed with H_2O (2 × 250 mL) and saturated aqueous NaCl (250 mL) and dried over $MgSO_4$. After the solvent was removed, 6.8 g (~100%) of crude product residue was distilled [bp 94–95 °C (22 torr)] to give 6.1 g (89.7%) of pure **9a**: NMR ($CDCl_3$) δ 0.67–1.10 (6 H, m, CH_3), 1.10–1.97 (10 H, m, CH_2), 2.3 [3 H, s, $CH_3C(=O)$], 3.48 (1 H, quintet, >CHS); IR (thin film) 1689 (>C=O) cm^{-1}. Anal. ($C_{10}H_{20}OS$) C, H, S.

***S*-Adenosyl-3-thiooctane (2b).** 3-Thioacetyloctane (**9a**; 480 mg, 2.55 mmol) in 10 mL of dry Me_2SO was degassed with a stream of nitrogen for 1 h, after which time 485 mg (1.7 mmol) of 5′-deoxy-5′-chloroadenosine (**5a**) was added, followed by 2 mL of 4 M NaOH. The resulting solution was stirred at ambient temperature overnight. The reaction mixture was then poured into 175 mL of H_2O to give a milky cloudy solution, which was cooled at −20 °C. After the solution was warmed to ~25 °C, a solid was collected by filtration to give 600 mg (88.8%) of crude **2b**. The crude product was recrystallized from MeOH–H_2O to give 550 mg (81.4%) of pure **2b**: mp 77–80 °C; NMR (CD_3OD) δ 0.5–0.95 (6 H, m, CH_3), 0.95–1.76 (10 H, m, CH_2), 2.48 (1 H, quintet, >CHS), 2.76 (2 H, d, $H_{5'}$, J = 6 Hz), 3.9–4.37 (2 H, m, $H_{3'}$ and $H_{4'}$) 5.88 (1 H, d, $H_{1'}$, J = 5 Hz), 8.06 (1 H, s, H_2), 8.16 (1 H, s, H_8), $H_{2'}$ peak obscured by OH signal (δ 4.33–5.1); UV λ_{max} 219, 260 nm; TLC R_f 0.93 (cellulose; BAW). Anal. ($C_{16}H_{29}N_5O_3S$) C, H, N, S.

***S*-Adenosyl-3-(methylthio)octane (3b). 2b** (160 mg, 0.4 mmol) was methylated in 1.5 mL of 88% formic acid with 0.5 mL of methyl iodide. The resulting solution was stirred at ambient temperature, protected from the light, for 3 days, after which time the reaction mixture was partitioned between 50 mL each of ether and H_2O. The aqueous layer was separated, washed with ether (3 × 50 mL), and lyophilized to give 150 mg (61.9%) of the iodide salt: NMR (D_2O) δ 0.6–1.5 (12H, broad complex, CH_3 and CH_2), 1.5–2.05 [4 H, broad complex, $CH_2C(S^+)CH_2$], 3.66 (1 H, m >CHS^+<), 3.66–4.03 (2 H, br m, $H_{5'}$), 6.15 (1 H, d, $H_{1'}$), 8.25 (1 H, s, H_2), 8.33 (1 H, s, H_8), $H_{2'}$, $H_{3'}$, and $H_{4'}$ peaks obscured by H_2O signal (4.0–5.4). The iodide was converted to a perchlorate salt by ion-exchange on a AG1-X8 column, to give pure **3b** as a white solid after lyophilization. The slightly hydroscopic product had mp 78–80 °C; UV λ_{max} 216, 259 nm; TLC R_f 0.7 and 0.84 [on cellulose developed with 5% aqueous Na_2HPO_4 and BAW (12:3:5), respectively]. Anal. ($C_{19}H_{32}N_5O_3S\cdot ClO_4$) C, H, N, S, Cl.

Route B. From 5′-Deoxy-5′-(thioacetyl)-*N*⁶-formyl-2′,3′-isopropylideneadenosine (4). Sodium methoxide (36 mg, 0.67 mmol) in 10 mL of dry methanol was degassed with a stream of nitrogen at ambient temperature for 1 h, and 225 mg (0.57 mmol) of **4** was added. The reaction was monitored by TLC (silica gel; EtOAc), which showed that the starting thioester **4** was converted to the thiol in ~10 min [R_f (SAc) 0.70 vs. R_f (SH and SS) 0.23 and 0.03 appeared). After this time, 210 mg (1.088 mmol) of 3-bromooctane (**8a**) was added, and a new compound (R_f 0.33) was formed after 2 h as shown by TLC. After the solution was continuously stirred at ambient temperature overnight, the solvent was removed to near dryness under reduced pressure, and the residue was partitioned between $CHCl_3$ (150 mL) and H_2O (100 mL). The organic layer was separated and washed with H_2O (100 mL), and saturated aqueous NaCl (100 mL) and dried over $MgSO_4$. After the solvent was removed, the crude product (220 mg) was purified on a preparative silica gel plate developed with EtOAc, the band at R_f 0.33 was removed, and the desired product was eluted with MeOH/$CHCl_3$ (1:1, 4 × 100 mL). The combined eluents were concentrated to near dryness, and the residue was redissolved in $CHCl_3$ and filtered. The filtrate was concentrated and dried under high vacuum to give 85 mg (34.3%) of pure thioether: NMR ($CDCl_3$) δ 0.67–1.01 (6 H, m, CH_3), 1.01–1.5 (10 H, m, CH_2), 1.33 (3 H, s, CH_3), 1.55 (3 H, s, CH_3), 2.68 (2 H, d, $H_{5'}$, J = 7 Hz), 2.4–2.9 (1 H, m, >CHS), 4.28 (1 H, dt, $H_{4'}$, J = 6 and 2 Hz), 4.95 (1 H, dd, $H_{3'}$, J = 2 and 7 Hz), 5.43 (1 H, dd, $H_{2'}$, J = 2 and 7 Hz), 5.98 (1 H, d, $H_{1'}$, J = 2 Hz), 6.21 (2 H, br s, NH_2), 7.81 (1 H, s, H_2), 8.2 (1 H, s, H_8). This thioether could be converted to **3b** by methylation with CH_3I in HCOOH, as described above for route A. The product **3b** obtained by route B was identical by NMR, mp, and TLC with that obtained by route A.

1,8-Dichloro-3-octanol (6b). A. 6-Chlorohexanoyl Chloride. Zinc chloride (2.26 g, 16.6 mmol) was added to 77.3 g (667 mmol) of ϵ-caprolactone in an ice bath to give a reddish solution to which 94.0 g (790 mmol) of thionyl chloride was added dropwise. After the addition, the color of the reaction mixture was a dark brown, which gradually became lighter in color as the reaction mixture was heated at 50–60 °C overnight. NMR spectra indicated that all the starting lactone had been consumed by this time. After the excess of thionyl chloride was removed under reduced pressure, the crude product was vacuum distilled [bp 70–72 °C (1.5 torr)] to give 57.49 g (51%) of a clear, colorless liquid: NMR ($CDCl_3$) δ 1.63 (6 H, m, CH_2), 2.88 [2 H, t, $CH_2C(=O)Cl$, J = 3.5 Hz), 3.57 (2 H, t, CH_2Cl, J = 3.0 Hz); IR (thin film) 1798 (>C=O) cm^{-1}. Anal. $C_6H_{10}Cl_2O$ (C, H).

B. 1,8-Dichloro-3-octanone. A well-stirred, ice-cooled solution of 30.35 g (179.6 mmol) of 6-chlorohexanoyl chloride in 300 mL of dry CCl_4 was degassed with nitrogen for 0.5 h. $AlCl_3$ (26.34 g, 179.6 mmol) was added in portions, and then ethylene was bubbled in at a rate so that no excess ethylene escaped from the reaction vessel. Ethylene was allowed to bubble through the reaction mixture at 0 °C for 2 h and at ambient temperature overnight. The reaction mixture was poured into 800 mL of ice–water and extracted with chloroform (2 × 400 mL). The combined chloroform extracts were washed with saturated aqueous $NaHCO_3$ (500 mL), H_2O (500 mL), and saturated NaCl (500 mL) and dried over $MgSO_4$. After the solvent was removed, 15.2 g (43%) of crude 1,8-dichlorooctanone was obtained, which was used without further purification: NMR ($CDCl_3$) δ 1.44 (6 H, m, CH_2), 2.34 [2 H, t, $CH_2C(=O)$, J = 3.0 Hz], 2.85 (2 H, t, $CH_2C(=O)$, J = 3.0 Hz), 3.52 (2 H, t, CH_2Cl, J = 3 Hz), 3.74 (2 H, t, CH_2Cl, J = 3.0 Hz).

C. 1,8-Dichloro-3-octanol (6b). To a well-stirred solution of 15.08 g (77 mmol) of 1,8-dichloro-3-octanone in 15 mL of 95%

ethanol, cooled in an ice bath, was added a solution of 1.97 g (52 mmol) of sodium borohydride in 7 mL of H_2O. Concentrated NH_4OH (20 mL) was added, and the resulting solution was stirred at ambient temperature for 1 h after the ice bath was removed. The reaction mixture was poured into 350 mL of H_2O and extracted with $CHCl_3$ (2 × 350 mL). The combined extracts were washed with 5% HCl solution (300 mL) and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the residue was vacuum distilled [bp 95–97 °C (0.3 torr)] to give 10.49 g (69%) of pure **6b**: NMR ($CDCl_3$) δ 1.42 (6 H, br m, CH_2), 1.85 [4 H, m, $CH_2C(OH)CH_2$], 2.24 (1 H, br s, OH, exchangable), 3.33–3.9 (5 H, overlapped multiplet, CH_2Cl and >CHO); IR (thin film) 3356 (OH) cm^{-1}. Anal. ($C_8H_{16}Cl_2O$) C, H, Cl.

1,8-Diphthalimido-3-octanol (6c). A solution of 4.87 g (45 mmol) of 1,8-dichloro-3-octanol (**6b**) and 9.97 g (54 mmol) of potassium phthalimide in 40 mL of dry DMF was heated at 100 °C for 2 h. As the reaction proceeded, the potassium phthalimide was slowly drawn into the solution to produce a slightly greenish color, and a fine white precipitate formed, presumably KCl. After cooling, the reaction mixture was partitioned between 300 mL each of $CHCl_3$ and H_2O. The organic layer was separated and washed with H_2O (300 mL), 1 N NaOH (300 mL), and H_2O (300 mL) and dried over $MgSO_4$. After the solvent was removed under reduced pressure, an oily residue was obtained, which crystallized upon trituration with ether. The solid was collected by filtration and recrystallized from MeOH–Et_2O to give 6.26 g (61%) of pure **6c** as long needle-shaped crystals: mp 144.5–145.5 °C; TLC R_f 0.35 (silica gel; MeOH/$CHCl_3$, 1:24); NMR ($CDCl_3$) δ 1.0–2.01 (10 H, complex, CH_2), 2.75 (1 H, br s, OH), 3.25–4.01 (5 H, m, $CH_2N<$ and >CHO), 7.71 (8 H, br s, aromatic). Anal. ($C_{24}H_{24}N_2O_5$) C, H, N.

1,8-Diphthalimido-3-(tosyloxy)octane (7c). To a solution of 3.0 g (7.1 mmol) of 1,8-diphthalimido-3-octanol (**6c**) in 15 mL of dry pyridine, cooled in an ice bath, was added in portions 5.63 g (29.5 mmol) of recrystallized tosyl chloride to give a brownish solution. After ~10 min at 0 °C, a white precipitate, presumably pyridinium hydrochloride, formed, and the resulting mixture was stirred overnight at 4 °C. The mixture was then poured into 300 mL of ice–water and extracted with $CHCl_3$ (2 × 200 mL). The combined organic extracts were washed with cold 1 N HCl (3 × 100 mL), and saturated aqueous NaCl (150 mL) and dried over $MgSO_4$. After the solvent was removed under reduced pressure, a yellow oily residue was obtained, which gave a white solid after trituration several times with ether and cooling to −20 °C for several hours. The solid thus isolated [3.56 g (87%); R_f 0.60 (silica gel; MeOH/$CHCl_3$, 1:24] was used without any further purification: NMR ($CDCl_3$) δ 1.12–2.10 (6 H, m, CH_2), 1.97 [4 H, m, $CH_2C(OH)CH_2$], 2.40 (3 H, s, CH_3), 3.35–3.86 (4, H, m, $CH_2N<$), 4.66 (1 H, m, >CHO), 7.11–8.07 (12 H, br s and partially obscured AA′BB′, aromatic).

1,8-Diphthalimido-3-bromooctane (8c). To a solution of 3.56 g (6.2 mmol) of crude tosylate **7c** in 125 mL of dry Me_2SO was added 17.1 g (166 mmol) of previously dried sodium bromide, and the resulting solution was stirred at ambient temperature for 2 days. The reaction was monitored by TLC on silica gel, MeOH/$CHCl_3$ (1:24), which indicated that the starting tosylate **7c** was consumed during this time. The mixture was poured into $CHCl_3$ (100 mL), washed with 10% H_2SO_4 (2 × 250 mL), and saturated aqueous NaCl (100 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the yellow oily residue was triturated with ether to yield a white solid, which after several recrystallizations from MeOH gave 0.975 g (33%) of pure **8c**: mp 107–109 °C; TLC R_f 0.70 (silica gel; MeOH/$CHCl_3$, 1:24); NMR ($CDCl_3$) δ 1.12–2.10 (6 H, m, CH_2), 1.97 [4 H, m, $CH_2C(OH)CH_2$], 3.35–3.86 (5 H, m, $CH_2N<$ and >CHO), 7.71 (8 H, br s, aromatic). Anal. ($C_{24}H_{23}BrN_2O_4$) C, H, N.

1,8-Diphthalimido-3-(thioacetyl)octane (9c). The bromide **8c** (400 mg, 0.828 mmol) was reacted with 144 mg (1.26 mmol) of previously triturated potassium thioacetate in 5 mL of dry Me_2SO at ambient temperature. After workup as described previously for **9a**, the oily residue was triturated with MeOH to give 278 mg (70%) of **9c** as a chromatographically pure solid, mp 120.5–122 °C. The analytical sample was recrystallized from hot MeOH: NMR ($CDCl_3$) δ 1.06–2.1 (10 H, complex, CH_2), 2.2 [3 H, s, $CH_3C(=O)$], 3.36–3.9 (4 H, m, $CH_2N<$), 7.6 (8 H, br s, aromatic). Anal. ($C_{26}H_{26}N_2O_5S$) C, H, N, S.

***N,N′*-Bis(*tert*-butoxycarbonyl)-3-hydroxy-1,8-octanediamine (6d). A. 1,8-Diiodo-3-octanol.** A mixture of 32 g (213 mmol) of NaI, previously dried at 110 °C overnight in an oven, and 20 g (100 mmol) of 1,8-dichloro-3-octanol (**6b**) in 240 mL of dry 2-butanone was refluxed on a steam bath for 2 days with the aid of an overhead mechanical stirrer. After NaCl was removed by filtration, the filtrate was concentrated under reduced pressure, and the oily residue was dissolved in 400 mL of $CHCl_3$, washed with 5% $Na_2S_2O_3$ (2 × 400 mL), saturated aqueous $NaHCO_3$ (2 × 400 mL), and H_2O (400 mL), and dried over $MgSO_4$. After removal of the solvent and drying under high vacuum, 30.57 g (80%) of practically pure diiodooctanol was obtained, which was used without further purification: NMR ($CDCl_3$) δ 1.36 (6 H, br m, CH_2), 1.86 [4 H, m, $CH_2C(OH)CH_2$], 2.56 (1 H, s, OH), 3.2 (4 H, q, CH_2I), 3.63 (1 H, m, >CHO).

B. *N,N′*-Bis(*tert*-butoxycarbonyl)-*N,N′*-bis(methoxycarbonyl)-3-hydroxy-1,8-octanediamine. Methyl *tert*-butyl iminodicarboxylate potassium salt[15] (1.3 g, 6.10 mmol) was suspended in a solution of 1.1 g (2.88 mmol) of 1,8-diiodo-3-octanol in 8 mL of dry DMF and heated at 75 ± 5 °C for 2 days, during which time solid KCl precipitated. The reaction mixture was then partitioned between 100 mL each of $CHCl_3$ and H_2O. The $CHCl_3$ layer was separated, washed with H_2O (4 × 100 mL), and dried over $MgSO_4$. After removal of the solvent and drying under high vacuum, 1.40 g (96.8%) of product was obtained as a chromatographically pure yellow oil, which was used without any further purification: NMR ($CDCl_3$) δ 0.98–1.96 (28 H, complex, CH_2 and CH_3), 3.13 (2 H, t, $CH_2N<$, J = 5 Hz), 3.6 (2 H, t, $CH_2N<$, J = 8 Hz), 3.73 (6 H, s, CH_3O), 4.06 (1 H, br s, OH), 4.53–5.1 (1 H, m, >CHO); IR (thin film) 3401 (OH) 1782 (>C=O), 1739 (>C=O) cm^{-1}.

C. *N,N′*-Bis(*tert*-butoxycarbonyl)-3-hydroxy-1,8-octanediamine (6d). A solution of 1.3 g (2.73 mmol) of *N,N′*-bis(*tert*-butoxycarbonyl)-*N,N′*-bis(methoxycarbonyl)-3-hydroxy-1,8-octanediamine in 8 mL of 1 N NaOH and 6 mL of methanol was stirred at ambient temperature overnight. The reaction mixture was then diluted with 60 mL of H_2O and then extracted with ether (3 × 60 mL). The combined ether extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give 800 mg of crude product, which after being triturated several times with petroleum ether gave 520 mg (52.9%) of pure **6d** as a solid: mp 66–68 °C; NMR ($CDCl_3$) δ 1.05–1.81 (28 H, complex, CH_2 and CH_3), 2.78–3.3 (3 H, m, $CH_2N<$ and >CHO), 3.3–3.81 (3 H, m, $CH_2N<$ and OH), 4.51–5.0 (1 H, br, NH), 5.0–5.4 (1 H, br, NH). Anal. ($C_{18}H_{36}N_2O_5$) C, H, N.

1,8-Diamino-3-octanol (6g). *N,N′*-Bis(*tert*-butoxycarbonyl)-3-hydroxy-1,8-octanediamine (**6d**; 360 mg, 1 mmol) in 3 mL of trifluoroacetic acid was stirred at ambient temperature for 2 h. The excess trifluoroacetic acid was then removed under high vacuum at ambient temperature, and the residue was dissolved in 15 mL of H_2O, washed with ether (3 × 15 mL), and lyophilized to give 372 mg (96%) of pure **6g** as an oily residue: TLC R_f 0.35 (silica gel; 1-propanol/NH_3/H_2O, 6:8:1); NMR (D_2O) δ 1.0–2.16 (10 H, complex m, CH_2), 2.66–3.23 (3 H, m, $CH_2N<$ and >CHO), 3.58 (2 H, t, $CH_2N<$). For analysis, a sample of **6g** was converted to its hydrochloride salt on a AG1-X8 column and lyophilized, and the residue was recrystallized from EtOH–Et_2O, mp 122–124 °C. Anal. ($C_8H_{20}N_2O$·HCl) C, H, N.

***N,N,N′,N′*-Tetrakis(*tert*-butoxycarbonyl)-3-hydroxy-1,8-octanediamine (6e).** Sodium hydride (1.2 g), 57%, in wax was washed three times with dry benzene, the benzene was removed by decantation, and the residual was NaH decanted and dried under high vacuum to remove the last traces of solvent: yield 820 mg (34 mmol) of sodium hydride. To a suspension of this sodium hydride (820 mg, 34 mmol) in 40 mL of dry DMF was added 6.2 g (28.3 mmol) of di-*tert*-butyliminodicarboxylate.[15] After the mixture was vigorously stirred overnight at 60 °C, a catalytic amount of lithium iodide was added, followed by 2.84 g (14.1 mmol) of 1,8-dichloro-3-octanol (**6b**), and the resulting solution was heated at 60 °C continuously for another 3 days. The reaction mixture was cooled, diluted with 400 mL of H_2O, and extracted with $CHCl_3$ (2 × 400 mL). The combined chloroform extracts were washed with H_2O (3 × 200 mL) and saturated aqueous NaCl solution (2 × 200 mL) and dried over $MgSO_4$. After removal of the solvent under reduced pressure and drying under high vacuum, 6.94 g (87.6%) of **6e** was obtained as a chromato-

graphically pure oil: NMR ($CDCl_3$) δ 1.09–2.1 (46 H, complex, CH_2 and CH_3), 3.56 (4 H, m, $CH_2N<$), 4.96 (1 H, m, >CHO). For further characterization, this product, **6e**, was converted to 1,8-diamino-3-octanol (**6g**) by treatment with CF_3COOH: TLC (silica gel; 1-propanol/NH_3/H_2O, 6:8:1) and NMR were identical with those of **6g** prepared from **6d** as described above.

***N,N,N',N'*-Tetrakis(*tert*-butoxycarbonyl)-3-(tosyloxy)-1,8-octanediamine (7e).** To a solution of 5.22 g (9.3 mmol) of *N,N,N',N'*-tetrakis(*tert*-butoxycarbonyl)-3-hydroxy-1,8-octanediamine (**6e**) in 27 mL of dry pyridine, cooled in an ice bath, was added 7.9 g (41.4 mmol) of recrystallized tosyl chloride in portions, and the resulting solution was stirred at 4 °C for 3 days. The reaction mixture was then poured into 500 mL of ice–water and extracted with ether (3 × 270 mL). The combined ether extracts were washed with 5% cupric chloride solution to remove pyridine and with saturated aqueous NaCl solution (3 × 200 mL) and dried over $MgSO_4$. After the removal of the solvent under reduced pressure and drying under high vacuum, 5.29 g (79.5%) of **7e** was obtained as an oil: TLC R_f 0.77 (silica gel; EtOAc/$CHCl_3$, 1:4); NMR ($CDCl_3$) δ 1.03–2.13 (46 H, complex, CH_2 and CH_3), 2.46 (3 H, s, CH_3), 3.50 (4 H, m, $CH_2N<$), 4.73 (1 H, m, >CHO), 7.23–7.96 (4 H, AA'BB', aromatic).

***N,N,N',N'*-Tetrakis(*tert*-butoxycarbonyl)-3-bromo-1,8-octanediamine (8e).** *N,N,N',N'*-Tetrakis(*tert*-butoxycarbonyl)-3-(tosyloxy)octanediamine (**7e**; 2.68 g, 3.74 mmol) in 20 mL of dry Me_2SO was treated with 1.6 g (18.4 mmol) of previously dried lithium bromide, and the resulting solution was stirred at ambient temperature for 3 days. The reaction mixture was then poured into 200 mL of $CHCl_3$, washed with H_2O (2 × 140 mL) and saturated aqueous NaCl solution (2 × 140 mL), and dried over $MgSO_4$. After removal of the solvent under reduced pressure and drying under high vacuum, 2.24 g (95.8%) of **8c** was obtained as a very viscous oil: NMR ($CDCl_3$) δ 1.13–2.06 (4 H, complex, CH_2 and CH_3), 3.45 (4 H, m, $CH_2N<$), 4.66 (1 H, m, >CHBr); IR (thin film) 1730 (C=O) cm^{-1}.

***N,N,N',N'*-Tetrakis(*tert*-butoxycarbonyl)-*S*-(2',3'-isopropylideneadenosyl)-1,8-diamino-3-thiooctane.** Sodium methoxide (59 mg, 1.09 mmol) in 11 mL of dry methanol was degassed with a stream of nitrogen for 1 h at ambient temperature, and 271 mg (0.688 mmol) of 5'-(thioacetyl)-5'-deoxy-N^6-formyl-2',3'-isopropylideneadenosine (**4**) was added. The reaction was monitored by TLC (silica gel; EtOAc) as described above for the reaction of **4** and **8a**. After 10 min, 420 mg (0.688 mmol) of *N,N,N',N'*-tetrakis(*tert*-butoxycarbonyl)-3-bromo-1,8-octanediamine (**8e**) was added, and a new compound (R_f 0.38) was formed after 2 h as shown by TLC. After the solution was continuously stirred at ambient temperature overnight, the solvent was removed to near dryness under reduced pressure, and the residue was partitioned between 150 mL of $CHCl_3$ and 100 mL of H_2O. The organic layer was separated, washed with H_2O (2 × 100 mL) and saturated NaCl solution (100 mL), and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product (450 mg) was purified on a preparative silica gel plate developed with EtOAc, the band at R_f 0.38 was removed, and the desired product was eluted with MeOH/$CHCl_3$ (1:1; 4 × 100 mL). The combined eluents were concentrated to near dryness, and the residue was redissolved into $CHCl_3$ and filtered. The filtrate was concentrated and dried under high vacuum to give 100 mg (16.8%) of pure thioether as a solid: NMR ($CDCl_3$) δ 1.03–2.26 (52 H, complex, CH_2 and CH_3), 2.76 (2 H, d, $H_{5'}$, J = 6 Hz), 3.03 (1 H, m, >CHS), 3.60 (4 H, m, $CH_2N<$), 4.4 (1 H, m, $H_{4'}$), 5.03 (1 H, m, $H_{3'}$), 5.46 (1 H, m, $H_{2'}$), 5.86 (1 H, s, $H_{1'}$), 6.01 (2 H, s, NH_2), 7.83 (1 H, s, H_2), 8.25 (1 H, s, H_8).

***S*-Adenosyl-1,8-diamino-3-(methylthio)octane (3c).** *N,N,N',N'*-Tetrakis(*tert*-butoxycarbonyl)-*S*-(2',3'-isopropylideneadenosyl)-3-(thioacetyl)-1,8-octanediamine (84 mg, 97 μmol) in 2 mL of 88% formic acid was treated with 100 μL (228 mg, 1.6 mmol) of methyl iodide. The resulting solution, protected from light, was continuously stirred at ambient temperature for 3 days. The reaction was monitored by TLC (silica gel; EtOAc), which showed that a sulfonium salt (R_f 0.0) was formed, and the starting thioether was consumed after 1 day. The reaction mixture was then poured into H_2O (50 mL) and extracted with ether (3 × 50 mL). The aqueous layer was separated and lyophilized to give the sulfonium iodide: NMR (D_2O) δ 1.06–1.76 (6 H, br complex, CH_2), 1.76–2.36 [4 H, br complex, $CH_2C(S^+)CH_2$], 2.93 (3 H, s, CH_3S^+), 3.23–3.9 (5 br, and $CHS^+<$), 3.9–4.06 (2 H, br, $H_{5'}$), 8.36 (2 H, s, H_2 and H_8). The peaks for $H_{2'}$, $H_{3'}$, and $H_{4'}$ were obscured by the HOD signal (δ 4.0–5.4). The iodide thus 211, 260 nm; to the perchlorate salt by use of an AG1-X8 column to give 26 mg (40%) of pure **3c** as a white solid, which was very hygroscopic: UV λ_{max} 211, 260 nm, TLC R_f 0.74 (cellulose; 5% aqueous Na_2HPO_4).

Dimethyl(5'-adenosyl)sulfonium Perchlorate (3a). 5'-(Methylthio)adenosine (**2a**; 120 mg (4 mmol), prepared according to the method published by Coward et al.,[13] was methylated to give 140 mg (84.1%) of **3a** by the method described previously for *S*-adenosyl-3-(methylthio)octane (**3b**): mp 76 °C dec; NMR (D_2O) δ 2.96 [6 H, s, $S^+(CH_3)_2$], 3.85 (2 H, d, $H_{5'}$, J = 6 Hz), 4.45–4.75 (2 H, complex, $H_{3'}$ and $H_{4'}$), 4.8–5.08 (1 H, m, $H_{2'}$), 6.08 (1 H, d, $H_{1'}$, J = 5 Hz), 8.21 (1 H, s, H_2), 8.26 (1 H, s, H_8); UV λ_{max} 207, 259 nm. Anal. ($C_{12}H_{18}N_5O_3S\cdot ClO_4$) C, H, N, Cl, S.

1,8-Diazido-3-hydroxyoctane (6f). A mixture of 5.5 g (27.6 mmol) of 1,8-dichloro-3-octanol (**6b**), 5.5 g (84.57 mmol) of sodium azide, and a catalytic amount of anhydrous lithium iodide in 25 mL of dry DMF was heated to 60 ± 5 °C (oil bath) for 1 day and then stirred at ambient temperature for another day. The solvent was then removed to near dryness under vacuum, at a temperature of ~30 °C. The residue was then partitioned between 250 mL each of $CHCl_3$ and H_2O. The organic layer was separated and washed with H_2O (2 × 250 mL) and saturated aqueous NaCl (250 mL), and dried over $MgSO_4$. After the solvent was removed, the crude product was distilled [bp 105–106 °C (0.015 torr)] to give 5.25 g (89.7%) of pure **6f**: NMR ($CDCl_3$) δ 1.18–2.0 (10 H, complex, CH_2), 2.43 (1 H, br s, OH), 3.08–4.01 (5 H, complex, CHO and CH_2N_3); IR (thin film) 3448 (OH), 2105 (N_3) cm^{-1}. Anal. ($C_8H_{16}N_6O$) H; C: calcd, 45.27; found, 44.60; N: calcd, 39.59; found, 40.12.

1,8-Diazido-3-(tosyloxy)octane (7f). 1,8-Diazido-3-hydroxyoctane (**6f**; 4.24 g, 20 mmol) in 40 mL of dry pyridine was cooled in an ice bath, 16.9 g (88.89 mmol) of recrystallized tosyl chloride was added in portions, and the resulting solution was stirred at 4 °C for 1 day. A white precipitate, presumably pyridinium hydrochloride, was formed from a clear pink solution after several hours. The reaction mixture was poured into 750 mL of ice–water and extracted with $CHCl_3$ (3 × 250 mL). The combined $CHCl_3$ extracts were washed with H_2O (2 × 250 mL), cold 5% H_2SO_4 (2 × 250 mL), saturated aqueous $NaHCO_3$ (250 mL), and saturated aqueous NaCl (250 mL) and dried over $MgSO_4$. After removal of the solvent and drying under high vacuum, 9.92 g (~100%) of the crude product **7f** was obtained and used without any further purification: NMR ($CDCl_3$) δ 1.0–2.2 (10 H, complex, CH_2), 2.47 (3 H, s, CH_3), 3.03–3.66 (4 H, m, CH_2N_3), 4.68 (1 H, quintet, >CHO), 7.23–7.98 (4 H, AA'BB', aromatic); IR (thin film) 2109 (N_3) cm^{-1}.

1,8-Diazido-3-(thioacetyl)octane (9f). Previously triturated potassium thioacetate (3.43 g, 30 mmol) was added to a solution of 9.92 g (~20 mmol) of crude **7f** in 90 mL of dry Me_2SO, and the resulting solution was stirred at ambient temperature for 1 day. The reaction mixture was then poured into 500 mL of H_2O and extracted with $CHCl_3$ (3 × 200 mL). The combined $CHCl_3$ extracts were washed with H_2O (4 × 300 mL) and saturated aqueous NaCl (2 × 300 mL) and dried over $MgSO_4$. After the removal of the solvent and drying under high vacuum, 4.33 g (80.2%) of crude product was obtained. Vacuum distillation [bp 138–141 °C (0.25 torr)] gave 2.1 g (39%) of the pure **9f**: NMR ($CDCl_3$) δ 1.16–2.13 (10 H, complex, CH_2), 2.36 [3 H, s, C(=O)CH_3], 3.1–3.9 (5 H, complex, >CHS, CH_2N_3); IR (thin film) 2105 (N_3), 1687 (>C=O) cm^{-1}; TLC R_f 0.82 (silica gel; MeOH–$CHCl_3$, 1:4). Anal. ($C_{10}H_{18}N_6O$) C, N; H: calcd, 6.71; found, 7.35.

***S*-Adenosyl-1,8-diazido-3-thiooctane (2d).** Pure **9f** (688 mg, 2.55 mmol) was coupled with 5'-deoxy-5'-chloroadenosine (**5a**) by the procedure described previously for **2b** to give 541 mg (66.7%) of practically pure **2d**, which was recrystallized from MeOH–H_2O to give pure **2d**: mp 44–46 °C; TLC R_f 0.71 (silica gel; MeOH–$CHCl_3$, 1:4); HPLC: t_r 21.3 min (ODS-2, 65% aqueous MeOH); NMR (CD_3OD) δ 0.83–1.9 (10 H, complex, CH_2), 2.26–2.63 (1 H, m, >CHS), 2.63–2.9 (2 H, d, $H_{5'}$, J = 6 Hz), 2.9–3.53 (4 H, m, CH_2N_3), 3.83–4.4 (2 H, complex, $H_{3'}$ and $H_{4'}$), 5.91 (1 H, d, $H_{1'}$, J = 5 Hz), 8.1 (1 H, s, H_2), 8.16 (1 H, s, H_8), $H_{2'}$ peak obscured by OH signal (δ 4.36–4.85); IR (Nujol) 2118 (N_3) cm^{-1}; UV λ_{max} 210, 259 nm; TLC R_f 0.71 (silica gel; $CHCl_3$–MeOH, 4:1),

0.88 (cellulose; BAW). Anal. ($C_{18}H_{27}N_{11}O_3S$) N; C: calcd, 45.27; found, 44.72; H: calcd, 5.70; found, 6.61; S: calcd, 6.72; found, 7.27.

S-Adenosyl-1,8-diamino-3-thiooctane (2c). S-Adenosyl-1,8-diazido-3-thiooctane (**2d**; 280 mg, 0.49 mmol) and triphenylphosphine (420 mg, 1.6 mmol) were dissolved in 1 mL of dry pyridine, and the resulting solution was kept at ambient temperature with stirring for 1 h, during which time gas evolution (presumably N_2) was observed. Ammonium hydroxide (15 M, 300 μL) was then added, and stirring was continued for another 2 h. The excess ammonium hydroxide and pyridine were removed under high vacuum at room temperature, and the resulting residue was dissolved in 70 mL of H_2O. The aqueous solution was washed with benzene (3 × 50 mL) and ether (3 × 50 mL) and then lyophilized to give 212 mg (82.5%) of free aminonucleoside as a hygroscopic white solid: NMR (D_2O) δ 0.67–1.96 (10 H, br, CH_2), 2.3–3.06 (7 H, complex, $CH_2N<$, >CHS, and $H_{5'}$), 4.06–4.4 (2 H, complex, $H_{3'}$ and $H_{4'}$), 5.91 (1 H, d, $H_{1'}$, J = 5 Hz), 8.03 (1 H, s, H_2), 8.16 (1 H, s, H_8). The peak of $H_{2'}$ was obscured by the HOD signal (δ 4.43–5.06): UV λ_{max} 212, 259 nm; TLC R_f 0.18 and 0.57 (developed with BAW on silica gel and cellulose plate, respectively), 0.71 (cellulose; 5% Na_2HPO_4). Anal. ($C_{18}H_{31}N_7O_3S$) C, H, N.

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Note Added in Proof: In collaboration with Professor Anthony Pegg, we have recently shown that compounds of the type described in this paper have profound effects on polyamine biosynthesis in cultured mammalian cells. As predicted from the data presented in Table I, compound **2c** markedly inhibits the biosynthesis of spermidine, with associated accumulation of putrescine. Details of these findings will be published separately.

Mesoionic Purinone Analogues as Inhibitors of Cyclic-AMP Phosphodiesterase: A Comparison of Several Ring Systems[1]

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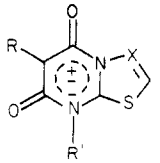
Several simple alkyl and aralkyl derivatives of mesoionic thiazolopyrimidines (1) and mesoionic 1,3,4-thiadiazolopyrimidines (2) were found to possess theophylline-like activity as inhibitors of cyclic-AMP phosphodiesterase (PDE). Reduction of the C_2–C_3 double bond of 1 or replacement of the sulfur atom of 1 or 2 with an *N*-methyl group nearly abolishes activity. Optimal activity appears to be associated with a hydrophobic substituent at the N_8 position. The five-membered ring of 1 can be replaced by a pyridine or isoquinoline nucleus without untoward effects. Preliminary kinetic data suggest that the type of enzyme inhibition produced by the mesoionic derivatives is similar to that observed for theophylline. Thus, several novel mesoionic ring systems display activity as inhibitors of cyclic-AMP PDE and can serve as lead compounds for further investigation.

Adenosine 3′,5′-monophosphate (cyclic-AMP) phosphodiesterase (PDE) is the enzyme responsible for the conversion of cyclic AMP to adenosine 5′-monophosphate and is also responsible, in part, for the regulation of intracellular levels of this cyclic nucleotide. An earlier communication from this laboratory reported that several derivatives of the mesoionic thiazolopyrimidines 1 and the



1, X = CH
2, X = N

mesoionic 1,3,4-thiadiazolopyrimidines 2 showed theophylline-like activity as inhibitors of cyclic-AMP PDE.[3] The potencies of these compounds were slightly less than that of theophylline; however, they do represent a novel class of PDE inhibitors.

Theophilline itself is not a particularly potent or selective PDE inhibitor; however, substituent alternation and molecular modification of the xanthine nucleus of theophylline has resulted in more potent derivatives.[4-6] Prior to a study directed toward the optimization of activity, or search for tissue/enzyme specificity, by manipulation of substituent groups (as has been done with the xanthines), it is necessary to determine whether or not the ring systems themselves are suitable templates. The primary objectives of this current study are (a) to compare the activities of a few examples of several different mesoionic purinone-related ring systems and (b) to attempt to show, for one ring system, that activity can be enhanced by varying substituent groups.

Chemistry. A number of the final products were prepared as we have previously reported.[3,7-10] This same

(1) This is the second in a series of four publications. The first, third, and fourth papers (ref 3; *J. Med. Chem.* **1981**, *24*, 658; and *Ibid.* **1981**, *24*, 766, respectively) have already appeared.
(2) Recipients of A. D. Williams Undergraduate Research Fellowships.
(3) Glennon, R. A.; Rogers, M. E.; Bass, R. G.; Ryan, S. B. *J. Pharm. Sci.* **1978**, *67*, 1762.
(4) Wells, J. N.; Wu, Y. J.; Baird, C. E.; Hardman, J. G. *Mol. Pharmacol.* **1975**, *11*, 1975.
(5) Goodsell, E. B.; Stein, H. H.; Wenzke, K. J. *J. Med. Chem.* **1971**, *14*, 1202.
(6) Garst, J. E.; Kramer, G. L.; Wu, Y. J.; Wells, J. N. *J. Med. Chem.* **1976**, *19*, 499.
(7) Coburn, R. A.; Glennon, R. A. *J. Heterocycl. Chem.* **1973**, *10*, 487.

0022-2623/81/1824-1284$01.25/0