medium was removed, and the monolayer was washed by dipping the dish into four successively 800-mL washes of cold 0.9% NaCl. The cells were then quantitatively removed from the dish, and aliquots of the suspension were used for determining total uptake. The distribution of radioactivity into acid-soluble and acid-insoluble cellular components was determined as follows. The cell suspension obtained above was diluted with trichloroacetic acid to a final concentration of 10%. After 3 h at 0 °C, the suspension was centrifuged. The supernatant solution containing the acid-soluble radioactivity was removed. The pellet was resuspended in 5% Cl_3AcOH and centrifuged. After the supernatant solution was removed, this procedure was repeated twice. The final pellet was resuspended in 5% Cl_3AcOH heated at 82 ± 1 °C for 30 min. The suspension was then cooled to 0 °C. The supernatant solution containing the nucleic acid fraction was removed. The pellet was quantitatively transferred to a glass-fiber filter (Whatman GF/C, 2.4 cm) presoaked with 5% Cl_3AcOH. The pellet containing cell protein was washed, and the radioactivity was determined as described.[34] The protein fraction, which could be redissolved in 0.5% $NaDodSO_4$, 0.1 M sodium phosphate, pH 7.3, had a λ_{max} at 276 nm; $A^{1\%}_{1cm}$ = 11.0; $A_{280/260}$ = 1.55 indicative of less than 1% contamination with nucleic acid.[35] All the radioactivity associated with the protein fraction eluted in the void volume on a Sephadex G-25 column run in the presence of 0.5% $NaDodSO_4$.

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Supplementary Material Available: Tables of data for the X-ray study of $C_4H_6FNO_4 \cdot 2H_2O$, atomic coordinates, selected bond angles, anisotropic thermal parameters, hydrogen atom distances and angles, and observed vs. calculated structure factors (12 pages). Ordering information is given on any current masthead page.

Synthesis and Biochemical Properties of Chemically Stable Product Analogues of the Reaction Catalyzed by *S*-Adenosyl-L-methionine Decarboxylase

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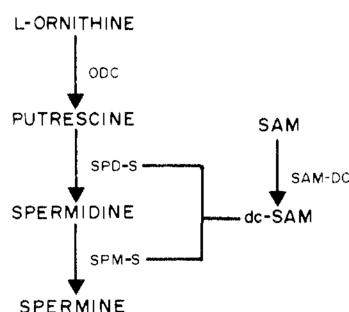
Structural analogues of decarboxylated *S*-adenosyl-L-methionine (dc-SAM), product of the reaction catalyzed by *S*-adenosyl-L-methionine decarboxylase (SAM-DC), with modifications in the side-chain portion of the molecule have been synthesized, and their ability to inhibit SAM-DC has been investigated. Mainly, compounds with a nitrogen atom in place of the sulfur were investigated. The data from these inhibition studies have resulted in a delineation of the structural features required for binding on SAM-DC. It was concluded that a terminal primary amino group, a terminal carboxyl group, and the sulfonium functionality are not required for binding on SAM-DC. It was also found that analogues of dc-SAM in which replacement of the sulfur by nitrogen was the only modification were still able to form an azomethine with the enzyme. As found for SAM and dc-SAM, these compounds also caused a time-dependent inactivation of SAM-DC.

Biosynthesis and accumulation of the polyamines putrescine, spermidine, and spermine appear intrinsically involved in cellular growth and proliferation.[1] In mammalian cells, polyamine biosynthesis involves the sequential action of two decarboxylases, L-ornithine decarboxylase (ODC; EC 4.1.1.17) and *S*-adenosyl-L-methionine decarboxylase (SAM-DC; EC 4.1.1.50), and two aminopropyltransferases, spermidine synthase (SPD-S; EC 2.5.1.16) and spermine synthase (SPM-S; EC 2.5.1.-) (Scheme I). Previous work[2] to develop effective inhibitors of polyamine biosynthesis in attempts to block the accumulation of polyamines in vivo was directed mainly toward L-ornithine decarboxylase. There are two reasons for this: first, the catalytic mechanisms of the aminopropyltransferases were not clarified until recently,[3] whereas the mechanism of action of pyridoxal phosphate dependent amino acid decarboxylases is well established;[4] second, L-ornithine decarboxylation was believed to be the rate-limiting step in the polyamine pathway[5] and, therefore, obviously the preferred target for inhibition of polyamine biosynthesis.

Scheme I



Only recently, *S*-adenosyl-L-methionine decarboxylase was investigated for the purpose outlined above. SAM-DC most probably is the rate-limiting enzyme in the biosynthesis of spermidine and spermine.[6] It differs from ODC

(1) J. Jänne, H. Pösö, and A. Raina, *Biochim. Biophys. Acta*, **473**, 241 (1978).
(2) (a) B. W. Metcalf, P. Bey, C. Danzin, M. J. Jung, P. Casara, and J. P. Vevert, *J. Am. Chem. Soc.*, **100**, 2551 (1978), and references cited therein; (b) H. G. Williams-Ashman and Z. N. Canellakis, *Perspect. Biol. Med.*, **22**, 421 (1979).
(3) K. Tang, A. E. Pegg, and J. K. Coward, *Biochem. Biophys. Res. Commun.*, **96**, 1371 (1980); V. Zappia, G. Cacciapuoti, G. Pontoni, F. Della Ragione, and M. Carteni-Farina, *Adv. Polyamine Res.*, **3**, 39 (1981), and references cited therein.
(4) E. A. Boeker and E. E. Snell, *Enzymes, 3rd Ed.*, **6**, 217 (1972).
(5) D. H. Russell and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420 (1968); J. Jänne and A. Raina, *Acta Chem. Scand.*, **22**, 1349 (1968); A. E. Pegg, D. H. Lockwood, and H. G. Williams-Ashman, *Biochem. J.*, 117, 17 (1970).

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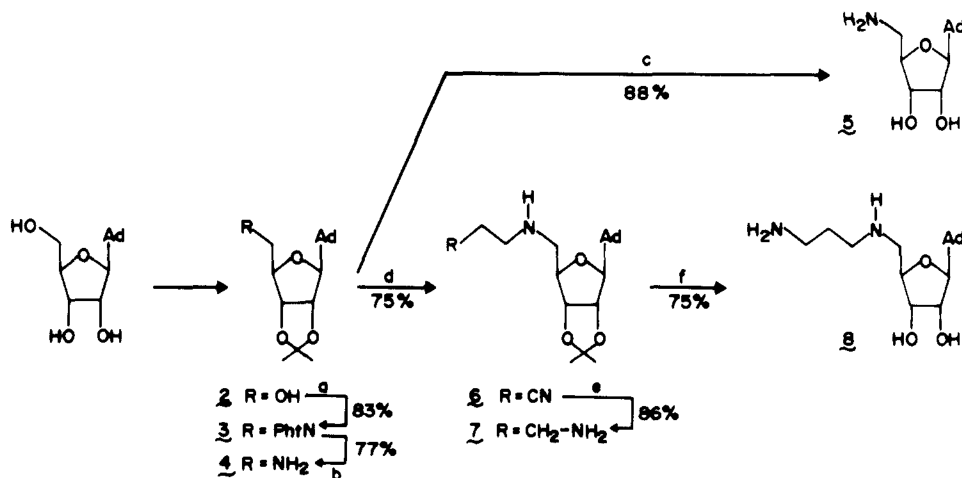
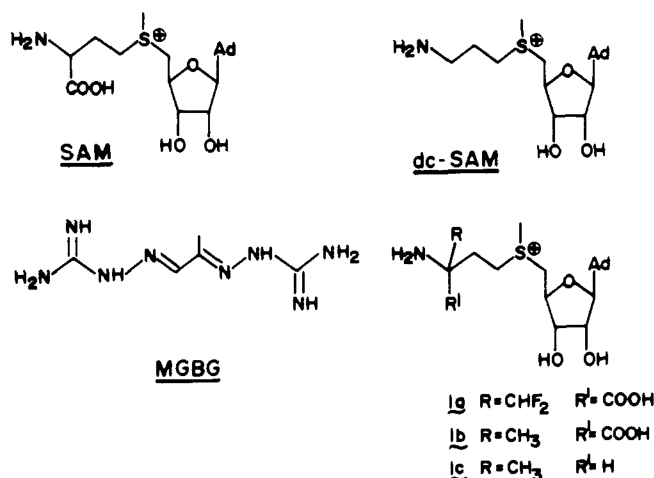
Scheme II[a,b]



[a] a, $EtO_2CN{=}NCO_2Et$, $P(C_6H_5)_3$, PhtNH; b, $H_2N{-}NH_2{\cdot}H_2O$; c, HCOOH; d, $NCCH{=}CH_2$; e, Ni(R), absolute $EtOH/NH_3$; f, 2 N HCl. [b] Ad = adenin-9-yl; PhtN = 1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl.

Chart I[a]



[a] Ad = adenin-9-yl.

in its mechanism of action in that it employs a pyruvoyl residue as the prosthetic group for catalytic activity.[1] Known inhibitors of SAM-DC are MGBG (Chart I) and some of its analogues.[7,8] These inhibitors, however, lack specificity;[8a,9] therefore, the possibilities of a more discriminating inhibition of SAM-DC were investigated.

Attempts to inhibit the activity of SAM-DC selectively by analogues of either its substrate (SAM) or the reaction product [decarboxylated SAM (dc-SAM)] are not new; recent examples are α-(difluoromethyl)-*S*-adenosylmethionine (**1a**), synthesized in our laboratory,[10] as well as compounds **1b** and **1c**[11] (Chart I). However, this approach has generally met with difficulties due to the inherent lability of adenosylsulfonium compounds,[12,13] a functionality reported "essential" for affinity toward SAM-DC.[14]

We have sought to overcome this problem by replacing the sulfur atom by a nitrogen atom. This substitution led to chemically stable compounds which, under physiological conditions, would still possess a cationic center at position 5′.[15] Here we report the synthesis and the biochemical properties of compounds **5**, **8**, **10**, **11**, **13**, **14**, and **17**, designed under this aspect: molecules **8**, **10**, **11**, **13**, and **14** as analogues of the product of the reaction catalyzed by SAM-DC, dc-SAM, a potent inhibitor of SAM-DC,[16] and compounds **5** and **17** by analogy with 5′-(dimethylsulfonio)-5′-deoxyadenosine (**15**),[13,17] a molecule found in our laboratory to inhibit SAM-DC activity quite efficiently (see Enzyme Inhibition Studies).

In the course of our investigations, an independent synthesis of compounds **11** and **13** was published.[11] However, the physical and biochemical properties reported for these two compounds differ considerably from our own findings, which therefore are documented here.

Results and Discussion

Chemistry. The synthesis of dc-SAM, racemic at sulfur,

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(9) N. E. Newton and M. M. Abdel-Monem, *J. Med. Chem.*, **20**, 249 (1977); E. Hölttä, P. Pohjanpelto, and J. Jänne, *FEBS Lett.*, **97**, 9 (1979); F. Mikles-Robertson, B. Feuerstein, C. Dave, and C. W. Porter, *Cancer Res.*, **39**, 1919 (1979); E. Höltta, H. Korpela, and T. Hovi, *Biochim. Biophys. Acta*, **677**, 90 (1981).

(10) P. Bey, J. P. Vevert, V. Van Dorsselaer, and M. Kolb, *J. Org. Chem.*, 44, 2732 (1979).

(11) M. Pankaskie and M. M. Abdel-Monem, *J. Med. Chem.*, **23**, 121 (1980).

(12) F. Schlenk, *Fortschr. Chem. Org. Naturst.*, **23**, 61 (1965).

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

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(15) J. Wagner, personal communication, Centre de Recherche Merrell International, Strasbourg, France, 1979: pK_a of 5′-N of compound **4** = 8.95; **8** = 11.45; **13** = 11.90, and **14** = 11.48.

(16) (a) K_I = 6.3 μM; B. Yamanoka and K. Samejima, *Chem. Pharm. Bull.*, **28**, 2232 (1980). (b) $K_I \approx 1$ μM; H. Pösö, R. Sinervirta, and J. Jänne, *Biochem. J.*, **151**, 67 (1975).

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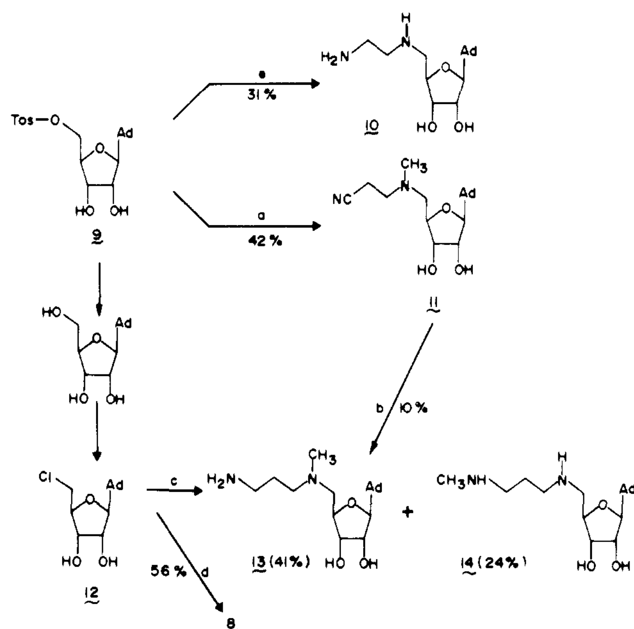
Scheme III[a,b]



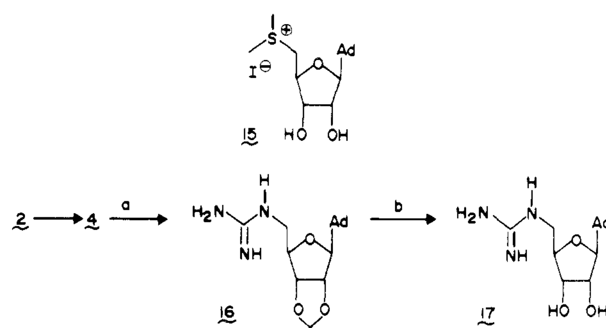
[a] a, $NC(CH_2)_2NHCH_3$; b, B_2H_6; c, $H_2N(CH_2)_3NHCH_3$; d, $H_2N(CH_2)_3NH_2$; e, $H_2N(CH_2)_2NH_2$. [b] Ad = adenin-9-yl; Tos = 4-(methylphenyl)sulfonyl.

followed closely the procedures described in the literature.[18,19] 5'-Chloro-5'-deoxyadenosine (12)[20] was used as starting material.

The target compound 8 was prepared as delineated in Scheme II. 2',3'-(1-Methylethylidene)adenosine (2), prepared[21] from adenosine, was reacted with triphenylphosphine, 1,3-dihydro-1,3-dioxo-2*H*-isoindole, and diethyl azodicarboxylate to give 5'-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-2',3'-(1-methylethylidene)-5'-deoxyadenosine (3).[22] Hydrazinolysis of 3 gave 5'-amino-2',3'-(1-methylethylidene)-5'-deoxyadenosine (4),[23] which was treated with 1 equiv of acrylonitrile to afford 5'-[(2-cyanoethyl)amino]-2',3'-(1-methylethylidene)-5'-deoxyadenosine (6). Hydrogenation of the nitrile in ethanol saturated with ammonia over Raney nickel catalyst gave 7, which after treatment with aqueous HCl afforded the target molecule 8 as its dihydrochloride salt. Attempts to prepare compound 13 (Scheme III) by a similar reaction pathway, i.e., the synthesis of 6 as described above, followed by treatment with methyl iodide and removal of the diol protecting group, failed due to a discouraging low yield in the methylating step. Therefore, alternative routes to 13 were investigated (Scheme III).

Treatment of 5'-[(4-methylphenyl)sulfonyl]adenosine (9) with 2-cyanoethylmethylamine gave 5'-[(2-cyanoethyl)methylamino]-5'-deoxyadenosine (11). However, subsequent hydroboration of the nitrile functionality resulted at best in a 10:1 mixture of 11 and 13, as judged from electrophoresis and HPLC (see Experimental Section). This stands in contrast to a synthesis reported[11] for 13,

Scheme IV[a,b]



[a] a, $H_2NC(NH)SEt$; b, H_2SO_4. [b] Ad = adenin-9-yl.

published during our investigations, which claimed that hydroboration was applied successfully in the conversion of 11 to 13. However, neither the published spectral and analytical data[11] nor the mass spectrum reported in the original thesis[24] allows one to judge conclusively the purity and structure of the material that was obtained. It is therefore suggested that the material described is a mixture of 11 and 13, which would then explain the differences in the reported[11,24] physical and biochemical properties compared to our material obtained as described below.

At this stage a more direct route to compounds 8 and 13 was developed (Scheme III). Reaction of 5'-chloro-5'-deoxyadenosine (12)[20] with an excess of diaminopropane at room temperature for 7 days afforded in 56% yield compound 8. By the same procedure, using 3-aminopropylmethylamine instead of diaminopropane, the target molecule 5'-[(3-aminopropyl)methylamino]-5'-deoxyadenosine (13) was obtained, which, however, was contaminated with 5'-[[3-(methylamino)propyl]amino]-5'-deoxyadenosine (14). The two N-methylated derivatives were separated by HPLC, and the correct position of the methyl group was established by mass spectroscopy. Both compounds give a molecular ion peak at m/e 338 (M + 1). The mass spectrum of compound 13 indicates a fragment M − 44 (m/e 293, M − $CH_2CH_2NH_2$), not detected for 14. On the other hand, the mass spectrum of 14 shows the fragmentation M − 58 (m/e 279, M − $CH_2CH_2NHCH_3$), which is not observed for 13. This feature therefore allows one to correlate the correct position of the methyl group in 13 and 14 with the fractions obtained from the HPLC separation.

5'-[(2-Aminoethyl)amino]-5'-deoxyadenosine (10), the next lower side-chain homologue of 8, was obtained by reaction of 9 with diaminoethane. 5'-Amino-5'-deoxyadenosine (5)[25] (Scheme II) and 5'-(dimethylsulfonio)-5'-deoxyadenosine iodide (15)[13] (Scheme IV) were prepared from 4 and 5'-(methylthio)-5'-deoxyadenosine, respectively, and had the expected melting points and spectral characteristics. Target molecule 17 (Scheme IV), reported only in the patent literature,[26] was synthesized by treatment of 4 with (ethylthio)uronium hydrobromide[27] in the presence of NaOH, affording 16. Removal of the isopropylidene protecting group with H_2SO_4 gave 5'-[(ami-

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(22) In analogy to O. Mitsunobu, S. Takizawa, and H. Morimato, *J. Am. Chem. Soc.*, **98**, 7858 (1976).
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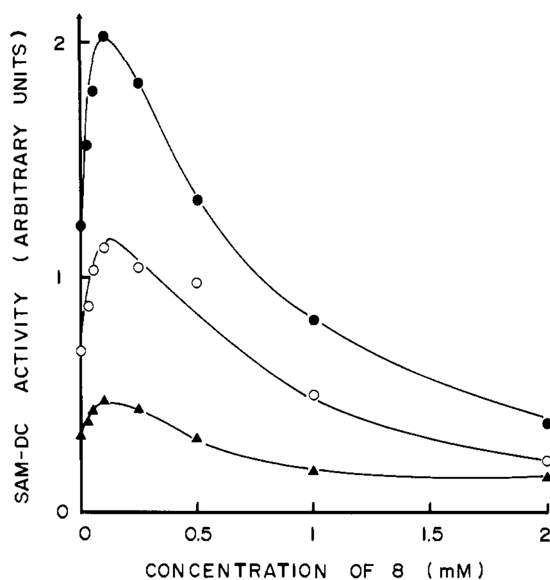

Figure 1. Effect of the concentration of 5′-[(3-aminopropyl)-amino]-5′-deoxyadenosine dihydrochloride (8) on the SAM-DC activity in the absence of putrescine at various concentrations of SAM: (▲) 20 μM; (○) 50 μM; (●) 100 μM.

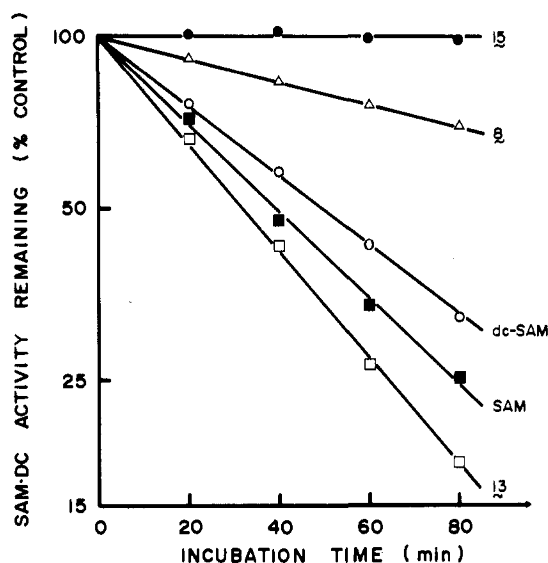

Figure 2. Time-dependent inhibition of SAM-DC by SAM analogues. The SAM-DC preparation was incubated at 37 °C in 30 mM, pH 7.1 sodium phosphate buffer, 5 mM dithiothreitol, with 1 mM inhibitor. At given time intervals, aliquots were withdrawn and assayed for remaining enzyme activity using *S*-adenosyl-L-[*carboxy*-^{14}C]methionine according to ref 28a.

Table I. Inhibition Constants of Various SAM Analogues toward SAM-DC

		inhibn constant (K_I), μM	
no.	inhibitor [a]	no putrescine	1 mM putrescine
	SAM	300	60
	dc-SAM	40	3 [d]
5	Ado-NH_2	1700	200
8	Ado-$NH(CH_2)_3NH_2$	*b*	50
10	Ado-$NH(CH_2)_2NH_2$	540	70
11	Ado-$N(CH_3)(CH_2)_2CN$	2800	300
13	Ado-$N(CH_3)(CH_2)_3NH_2$	380	60
14	Ado-$NH(CH_2)_3NHCH_3$	*c*	600
15	Ado-$S^+(CH_3)_2I^-$	20	2
17	Ado-NHC(NH)NH_2	1000	80

[a] Ado = 5′-deoxyadenosin-5′-yl. [b] Not determined; see Enzyme Inhibition Studies and Figure 1. [c] No inhibition at 1 mM. [d] Even if this value is in agreement with the data reported in the literature,[16] the real K_I could be lower lower due to the possibility of a contamination of the preparation of SAM-DC by SPD-S.

noiminomethyl)amino]-5′-deoxyadenosine sulfate (17·H_2SO_4).

Enzyme Inhibition Studies. Livers from MGBG-treated rats were used as a source of SAM-DC (see Experimental Section). The inhibition by the target compounds **5**, **8**, **10**, **11**, **13**, **14**, and **17** was studied in vitro in the absence and in the presence of putrescine (1 mM), since putrescine is an activator of SAM-DC.[16b,28] Examination of the Lineweaver–Burk[29] reciprocal plots of the data indicated that all target compounds and dc-SAM were competitive inhibitors of the enzymic decarboxylation reaction in the presence and, with the exception of **8**, in the absence of putrescine. In this particular situation, compound **8** was an activator at low concentrations, whereas it inhibited SAM-DC at high concentrations (Figure 1). The apparent inhibition constants of all compounds measured from a Dixon plot[30] are given in Table I.

Several conclusions concerning the features of the ligands essential for binding to the active site of SAM-DC can be drawn from these data: (1) The carboxyl group is nonessential for affinity, confirming previous results obtained from studies which show that dc-SAM has a fair affinity for the enzyme.[16] (2) The terminal primary amino group (and therefore the formation of an azomethine with the keto group of the pyruvoyl residue) is not an absolute prerequiste for affinity toward the active site, as exemplified by compounds **11**, **14**, **17**, and, particularly, **15**, the best inhibitor in this study. However, its relative importance for affinity is demonstrated by the differences of the K_I values of compounds **13** vs. **14** and **11**. (3) The sulfonium functionality, a third supposed binding-target group,[14] is not imperative for binding with SAM-DC. Replacement of the sulfur by a nitrogen atom still provided active inhibitors. This suggests that it is the charge at position 5′ which is important for binding, confirming a recent hypothesis of Pankaskie and Abdel-Monem.[11] (4) Putrescine appears to increase the affinity of the inhibitors whether or not the formation of an azomethine is possible. (5) Compound **8** is probably recognized by the enzyme as an analogue both of the activator putrescine and of the substrate. Figure 1 suggests that at least two molecules of **8** are able to bind together to the enzyme at two different sites. This result would favor the concept of an allosteric regulation of SAM-DC, whereas no demonstration of homotropic or heterotropic effects[31] had been obtained by using SAM or putrescine, respectively.[2b,16b,28b]

It has been reported by Pankaskie and Abdel-Monem[11] that incubation of SAM-DC with various concentrations of SAM resulted in a time-dependent loss of enzymic activity. We have confirmed this result (Figure 2) and, in addition, show here that preincubation of SAM-DC with dc-SAM or **8** or **13** gave similar enzyme inactivation, which could not be restored after a 24-h dialysis. Compounds

(28) (a) A. E. Pegg and H. G. Williams-Ashman, *J. Biol. Chem.*, **244**, 682 (1969); (b) P. S. Mamont and C. Danzin, *Adv. Polyamine Res.*, **3**, 123 (1981), and references cited therein.
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(31) J. Monod, J. Wyman, and J. P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).

Table II. Effects of Preincubation with Different Inhibitors on the Inactivation of SAM-DC [a]

addition to incubation media	time of half-inactivation of SAM-DC, [b] min
0.2 mM SAM	100 ± 7
0.2 mM SAM + 1 mM $NaBH_3CN$	25 ± 1
1 mM SAM	28 ± 1
1 mM SAM + 0.05 mM 15	77 ± 8
0.2 mM dc-SAM	100 ± 7
0.2 mM dc-SAM + 1 mM $NaBH_3CN$	10 ± 1
0.2 mM 8	250 ± 20
0.2 mM 8 + 1 mM $NaBH_3CN$	25 ± 3
0.2 mM 13	93 ± 10
0.2 mM 13 + 1 mM $NaBH_3CN$	12 ± 1
0.2 mM 13 + 0.05 mM 15 + 1 mM $NaBH_3CN$	50 ± 2
0.5 mM 13	41 ± 2
0.5 mM 13 + 0.05 mM 15	98 ± 4

[a] The experimental protocol is described in Figure 2. Under these conditions, the time of half-inactivation of SAM-DC alone in the incubation medium was >400 min. Addition of 1 mM $NaBH_3CN$ or of 0.05 mM of 15 or the combination of both did not alter further SAM-DC activity (i.e., the remaining activity of SAM-DC was >90% after an 80-min incubation). [b] Each value was calculated using a least-squares fit of the data points with a Hewlett-Packard 9820 calculator.

5, **10**, **11**, **14**, and **15**, having no primary terminal amino group or a shortened aliphatic side chain, did not inactivate the enzyme. Furthermore, compound **15**, which has a fair affinity for SAM-DC, protected efficiently against the enzyme inactivation by **13** (Table II), demonstrating the active-site-directed effect of these compounds. These results support the suggestion forwarded by Pankaskie and Abdel-Monem[11] that the inactivation of the enzyme would require a transamination of the inhibitor. Obviously further studies are necessary to confirm this hypothesis.

As reported in the literature,[11] we have also found that SAM-DC was inactivated upon preincubation with SAM and $NaBH_3CN$, a selective reducing agent of azomethines[32] (Table II). However, in our hands, in contrast to what is stated in the above-cited paper, compound **13** gave an analogous inactivating effect on SAM-DC (Table II), with no recovery of activity after a 24-h dialysis. This result demonstrates the formation of an azomethine of **13** with the prosthetic group of the enzyme. Therefore, we believe that **13** binds to the enzyme in a fashion analogous to that of the natural substrate SAM, contrary to what was reported.[11] Similar results were obtained with synthetic dc-SAM and **8** (Table II). The protective effect of the competitive inhibitor **15** (Table II) suggests further that this inactivation of SAM-DC is active site directed.

Experimental Section

Melting points were determined with a Buchi SMP-20 or a Kofler hot bank melting point apparatus and are uncorrected, as are the boiling points. ^1H NMR (60 MHz) spectra were recorded on a Varian Associates T-60 spectrometer and data are presented as follows: solvent, chemical shift, multiplicity, integration, coupling constants, and interpretation. ^{13}C NMR (22.63 MHz) spectra were recorded on a Brucker WH 90 spectrometer and are reported in parts per million downfield from tetramethylsilane. Electron-impact (EI; 70 eV) mass spectra were recorded on a Thomson THN 208, the sample was introduced via the direct introduction inlet, and source temperature was 230 °C. Desorption, coupled with chemical ionization (DCI)[33] were taken on a CG/SM Ribermag R10-10, NH_3. Infrared spectra were obtained on a Perkin-Elmer IR-577 or IR-277 spectrophotometer and ultraviolet spectra were obtained on a Cary 118 instrument. HPLC system: 6000-A solvent-delivery system, U6K injector, μBondapak C_{18} column (10-μm particle size range, 30 × 3.9 mm i.d.); UV absorption was monitored at 280 nm with a Model 440 UV absorbance detector from Waters Associates, Milford, MA; for preparative separations, a Water PrepLC/System 500 liquid chromatograph with PrepKAP-500-C_{18} column was used. For electrophoresis, 20 × 20 cm commercial silica gel covered glass plates (silica gel 60, E. Merck; silica gel 1500, Schleicher & Schüll, Dassel, G.F.R.) were used along with pyridine acetic acid buffer, pH 4.8.[34] The cooled (0 °C) plate thin-layer electrophoresis equipment according to Pastuska (Camag, Muttenz, Switzerland) was used: 600 V, ~80 mA, 60-min runs were suitable. Microanalyses were conducted on a Perkin-Elmer 240 CHN analyzer. Cl^- was determined colorimetrically by "Merckotest Chlorid" [no. 3311, $Hg(NO_3)_2$/diphenylcarbazone]. Water content was measured by the method of Karl Fischer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Reactions were routinely followed by ^1H NMR analysis of aliquots or by thin-layer chromatography (TLC) analysis. For analytical TLC, Merck precoated silica gel 60F-254 plates (0.25-mm thick) were used; the spots were detected by irradiation with a Mineralight UVS 11 and by charring after spraying with vanillin–sulfuric acid.[35] Compounds containing amino groups were also detected with ninhydrin spray. Silica gel (Kieselgel 60, partical size 0.063–0.200 mm, Merck) was used for chromatography. *S*-Adenosyl-L-methionine chloride salt (grade II) used in this study was purchased from Sigma Chemical Co.

Reactions described as "run under nitrogen" employed a mercury bubbler arranged so that the system could alternatively be evacuated and filled with inert gas and left under a positive pressure.

5′-[(3-Aminopropyl)methyl-(*R*,*S*)-sulfonio]-5′-deoxyadenosine Dihydrogen Sulfate (dc-SAM). In analogy to the literature,[18,19] 5′-chloro-5′-deoxyadenosine (**12**,[20] 2.28 g, 8 mmol) was reacted with 3-(benzylthio)propylamine[18] (1.74 g, 8 mmol) in liquid ammonia (300 mL) in the presence of metallic sodium. Modified was the isolation procedure for the reaction product: After removal of the ammonia, water (20 mL) and then HCl (1 N) were added to the residue to neutralize to pH 7. The mixture was filtered and the filtrate was evaporated to dryness. Coevaporation with *i*-PrOH removed residual water from the solid, to which absolute MeOH was added. This mixture was filtered again, and the filtrate was concentrated to afford a solid, which was applied to an ion-exchange resin (Dowex WX4 50, NH_4^+). The column was washed with 1 L of water and 0.1, 0.5, and 6 N NH_4OH (1 L each). The latter fraction was concentrated in vacuo to afford a solid (1.1 g), which was dissolved in 1 N H_2SO_4 (14 mL). Addition of absolute EtOH (60 mL) gave a crystalline precipitate, which was filtered off and washed with a little absolute EtOH. The solid was dissolved in water (15 mL) and precipitated again with absolute EtOH (75 mL). Repeating this operation three times gave analytically pure 5′-[(3-aminopropyl)thio]-5′-deoxyadenosine dihydrogen sulfate: yield 1.2 g (33%); mp 176 °C (lit.[18] mp 180–183 °C). The conversion of this material into dc-SAM was achieved following a procedure described in the literature:[19] dc-SAM·$2H_2SO_4$·0.5EtOH, mp 145 °C (lit.[19] mp 149–151 °C).

5′-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-2′,3′-(1-methylethylidene)-5′-deoxyadenosine (3). Diethyl azodicarboxylate (18.20 g, 0.105 mol) was added dropwise to a magnetically stirred suspension of 2′,3′-(1-methylethylidene)adenosine (**2**;[21] 32.15 g, 0.105 mol), 1,3-dihydro-1,3-dioxo-2*H*-isoindole (15.40

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g, 0.108 mol), and triphenylphosphine (27.40 g, 0.105 mol) in dry THF (360 mL) under nitrogen. An exothermic reaction occurred, and after complete addition, an orange solution was obtained. Ten to fifteen minutes later, a white precipitate separated, and after an additional 2 h, the reaction mixture was filtered. The isolated solid was washed with 1 L of diethyl ether and dried overnight at 25 °C (0.1 mmHg) to give **3** as a white powder: yield 38.03 g (83%); mp 198 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 and 7.83 (2 s, 2, C_2 H and C_8 H), 7.8 (s, 4, Ar), 6.0 (s, 2, NH_2), 5.97 (d, 1, J = 2 Hz, $C_{1'}$ H), 5.5 (d of d, 1, J = 7 and 3 Hz, $C_{2'}$ H), 5.2 (d of d, 1, J = 3 and 7 Hz, $C_{3'}$ H), 4.5 (m, 1, $C_{4'}$ H), 3.97 (d, 2, J = 7 Hz, $C_{5'}$ H), 1.57 and 1.37 (2 s, 6, CH_3); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 168.8, 152.9, 141.2, 134.6, 132.2, 123.5, 90.8, 85.3, 84.5, 82.8, 39.8, 27.0, 25.3 ppm; IR ($CHCl_3$) 3410 (NH), 2980 (CH_3), 1780 and 1720 (imide), 1635 (adenine) cm^{-1}; UV (EtOH) λ_{max} 258.0 nm (ϵ 2000), 241.8 (2057); EIMS, m/e (relative intensity) 436 (M, 2), 421 (8), 302 (10), 218 (100), 164 (80), 160 (55), 136 (25), 135 (25); TLC (EtOH) R_f 0.52. Anal. ($C_{21}H_{20}N_6O_5$) C, H, N.

5′-Amino-2′,3′-(1-methylethylidene)-5′-deoxyadenosine (4). A mixture of **3** (28.3 g, 0.065 mol) and hydrazine hydrate (52 g, 1.04 mol) in EtOH (2 L) was refluxed overnight. The obtained solution was allowed to cool to room temperature and then filtered, and the filtrate was evaporated to dryness in vacuo. The residue was treated with H_2O (300 mL), and the mixture was acidified with glacial acetic acid (pH 4), and filtered. The filtrate was adjusted to pH 10 with aqueous 4 N NaOH. Extraction with $CHCl_3$ (4 × 500 mL), drying of the combined organic layers over $MgSO_4$, and removal of the solvents in vacuo gave **4**: yield 15.3 g (77%); mp 203 °C (lit.[25] mp 203–207 °C).

5′-Amino-5′-deoxyadenosine Dihydrochloride (5). In close analogy to a literature procedure[36] for the removal of the 1-methylethylidene protecting group from nucleosides, a solution of **4** (1.23 g, 4 mmol) in 50% aqueous formic acid (15 mL) was kept for 24 h at room temperature and then evaporated to dryness. Coevaporating the residue with EtOH several times, until all formic acid was removed, afforded a white powder. This material was treated with absolute EtOH (50 mL), the mixture was filtered, and absolute EtOH, saturated with HCl gas, was added to the solution to precipitate **5**·2HCl, a white solid: yield 1.2 g (88%); mp 159 °C dec (for the hydrochloride, 150 °C dec,[25] 154–155 °C dec[37]); ^{1}H NMR (D_2O) δ 8.43 (s, 2, C_2 H and C_8 H), 6.17 (d, 1, J = 5 Hz, $C_{1'}$ H), 4.9 and 4.6 [t and m, 3 (1:2), $C_{2'}$ H, $C_{3'}$ H, and $C_{4'}$ H], 3.6 (d, 2, J = 7 Hz, $C_{5'}$ H); IR (KBr) 3300 (NH_2), 3050, 1625 (adenine) cm^{-1}; UV (H_2O) λ_{max} 258 nm (ϵ 15 200); TLC ($EtOH/NH_4OH$, 70:30) R_f 0.48. Anal. ($C_{10}H_{16}Cl_2N_6O_3$) C, H, Cl; N: calcd, 24.78; found, 24.09.

5′-[(2-Cyanoethyl)amino]-2′,3′-(1-methylethylidene)-5′-deoxyadenosine (6). Freshly distilled acrylonitrile (1.12 g, 0.021 mol) was added to a solution of **4** (6.00 g, 0.020 mol) in H_2O (8 mL). The mixture was stirred at room temperature for 18 h and then concentrated in vacuo [20 °C (15 mmHg)] to yield crude **6**, contaminated with starting material and the biscyanoethylated compound. The crude product (7 g) was applied to a column of silica gel (70–230 mesh, 350 g) and eluted with chloroform/MeOH (95:5). Fractions containing **6** were pooled and evaporated in vacuo. The residue obtained was recrystallized from MeOH to give 5.3 g (75%) of **6**: mp 63 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 and 7.83 (2 s, 2, C_2 H and C_8 H), 5.9 (s, 2, NH_2), 5.93 (d, 1, J = 3 Hz, $C_{1'}$ H), 5.47 (d of d, 1, J = 3 and 7 Hz, $C_{2'}$ H), 5.03 (d of d, 1, J = 3 and 7 Hz, $C_{3'}$ H), 4.3 (m, 1, $C_{4'}$ H), 2.9 (m, 4, N-CH_2), 2.45 (t, 2, J = 6 Hz, CH_2CN), 2.1 (s, 1 NH), 1.60 and 1.37 (2 s, 6, CH_3); IR ($CHCl_3$) 3460, 3300 and 3160 (NH), 2980 (CH_3), 2930 (CH_2), 2830, 2240 (CN), 1630 (adenine) cm^{-1}; UV (EtOH) λ_{max} 258.5 nm (ϵ 14 190); EIMS, m/e (relative intensity) 360 (M, 3), 344 (4), 319 (5), 218 (10), 204 (15), 164 (30), 136 (100), 100 (25); TLC (MeOH) R_f 0.57, ($EtOH/NH_4OH$, 80:20) R_f 0.74. Anal. ($C_{16}H_{21}N_7O_3$) C, H, N.

5′-[(3-Aminopropyl)amino]-2′,3′-(1-methylethylidene)-5′-deoxyadenosine (7). A solution of 5′-[(2-cyanoethyl)amino]-2′,3′-(1-methylethylidene)-5′-deoxyadenosine (**6**; 2.0 g, 5.6 mmol) in absolute EtOH (10 mL) saturated with NH_3 was hydrogenated over freshly prepared Raney Nickel catalyst (~0.5 g) at 120 °C for 24 h. The reaction mixture was filtered to remove the catalyst, and the filtrate was concentrated in vacuo. The resulting viscous residue was dissolved in H_2O (10 mL) and applied to a column of ion exchange resin (Dowex WX4 50, NH_4^+). After the column was washed with H_2O and 0.1, 0.5, 1, and 2 N NH_4OH (500 mL each), **7** was eluted with 12 N NH_4OH (500 mL): yield 1.74 g (86%); ^{1}H NMR (D_2O) δ 8.17 and 8.10 (2 s, 2, C_2 H and C_8 H), 6.10 (d, 1, J = 3 Hz, $C_{1'}$ H), 5.5 (d of d, 1, J = 3 and 6 Hz, $C_{2'}$ H), 5.0 (d of d, 1, J = 3 and 6 Hz, $C_{3'}$ H), 4.4 (m, 1, $C_{4'}$ H), 2.9–2.0 (m, 6, N-CH_2), 1.60 and 1.47 (2 s, 6, CH_3), 1.5 (m, 2, $C_{2''}$ H). Used without further purification in the synthesis of **8**.

5′-[(3-Aminopropyl)amino]-5′-deoxyadenosine Dihydrochloride (8). Prepared from 7. A solution of **7** (1.7 g, 4.7 mmol) in aqueous 2 N HCl (15 mL) was stirred at room temperature for 3 h. The reaction mixture was basified (pH 8) with concentrated NH_4OH and concentrated in vacuo [30 °C (20 mmHg)]. The residue was dissolved in H_2O (5 mL) and applied to a column of ion-exchange resin (Dowex WX4 50, NH_4^+). The column was washed with H_2O and 0.05, 0.1, 0.2, and 0.5 N NH_4OH (100 mL each); the product was then eluted with 2 N NH_4OH (1 L). The product-containing fraction was evaporated to dryness in vacuo, and the residue was dissolved in absolute EtOH (10 mL). To this solution, EtOH (5 mL), saturated with HCl gas, was added to give a precipitate, which was separated, dried [50 °C (0.01 mmHg)] and recrystallized from aqueous EtOH (95%) to give **8**·2HCl: yield 1.39 g (75%); mp 209 °C dec; spectral data (^{1}H NMR, IR, and MS) were identical with the compound prepared from **12**.

Prepared from 12. A mixture of 5′-chloro-5′-deoxyadenosine[20] (**12**; 9.0 g, 0.032 mol) and 1,3-diaminopropane (46 g, 0.622 mol), protected from light and humidity, was stirred for 7 days at room temperature. The mixture was poured into diethyl ether (500 mL). Decantation of the ether layer left an oil, which was further purified by HPLC ($CH_3CO_2NH_4/HCO_2H$ eluant, 5 × 10^{-2} M, pH 5.3). The product-containing fractions were pooled, the solvent was removed in vacuo, and the residue was applied to a column of ion-exchange resin (Dowex WX4 50, NH_4^+). The column was washed with H_2O (5 L), and the product was eluted with 6 N NH_4OH. The eluate was concentrated to dryness [50 °C (20 mmHg)], and the residue was dissolved in absolute EtOH (50 mL). Absolute EtOH (10 mL), saturated with HCl gas, was added, and the precipitate was collected. Recrystallization from aqueous EtOH (95%) gave **8**·3HCl·2H_2O: yield 7.0 g (56%); mp 185–190 °C dec; ^{1}H NMR (D_2O) δ 8.23 and 8.13 (2 s, 2, C_2 H and C_8 H), 6.07 (d, 1, J = 5 Hz, $C_{1'}$ H), 4.8 and 4.6 (2 m, 3, $C_{2'}$ H, $C_{3'}$ H, and $C_{4'}$ H), 3.8–3.0 (m, 6, N-CH_2), 2.1 (m, 2, $C_{2''}$ H); ^{13}C NMR (D_2O) 152.7, 150.6, 147.7, 146.0, 121.7, 92.1, 82.2, 75.8, 73.8, 51.7, 47.5, 39.2, 26.1 ppm; IR (KBr) 3350 (NH), 1700, 1590 cm^{-1}; UV (H_2O) λ_{max} 258 nm (ϵ 13 000); EIMS, m/e (relative intensity) 325 (M + 1, 1), 279 (2), 237 (3), 194 (18), 178 (46), 164 (15), 136 (100), 135 (38), 129 (62); homogeneous on electrophoresis under the conditions described above. Anal. ($C_{13}H_{28}Cl_3N_7O_5$) C, N, Cl, H_2O; H: calcd, 6.02; found, 5.51.

5′-[(2-Aminoethyl)amino]-5′-deoxyadenosine Trihydrochloride Hydrate (10). A stirred mixture of **9** (1.0 g, 3 mmol) and 1,2-diaminoethane (5 mL), protected from light and humidity, was kept for 7 days at room temperature and then poured into diethyl ether (100 mL). After an additional 2 days, a solid had separated. The ether layer was decanted, and the solid was dissolved in absolute EtOH (10 mL). The addition of 10 mL of EtOH, saturated with HCl gas, resulted in a precipitate, which was isolated by filtration and then applied to an ion-exchange resin (Dowex WX4 50, NH_4^+). The column was washed with 2 L of water, with 1.5 L of 0.15 N NH_4OH, and then with 0.22, 0.30, and 0.45 N NH_4OH (1 L each). The two latter fractions were combined and concentrated to give a solid, which was dissolved in absolute EtOH. Addition of EtOH, saturated with HCl gas, afforded a white precipitate, which was recrystallized from absolute EtOH to yield 0.4 g (31%) of **10**·3HCl·H_2O: ^{1}H NMR (D_2O) δ 8.5 (s, 2, C_2 H and C_8 H), 6.27 (d, 1, J = 5 Hz, $C_{1'}$ H), 5.3–4.3 (m, 3, $C_{2'}$ H, $C_{3'}$ H, and $C_{4'}$ H), 3.7 (d, 2, J = 6 Hz, $C_{5'}$ H), 3.6 (narrow m, 4, N-CH_2CH_2-N); homogeneous on electrophoresis. Anal. ($C_{12}H_{24}Cl_3N_7O_4$) C, H, N, Cl, H_2O.

5′-[(2-Cyanoethyl)methylamino]-5′-deoxyadenosine Hemihydrate (11). A stirred mixture of **9** (2.95 g, 7 mmol) and

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2-cyanoethylmethylamine (20 mL) at room temperature was protected from light and humidity for 7 days. The mixture was poured into diethyl ether (500 mL), and the ether was decanted from the oil, which was further purified by chromatography (25 g of silica gel; $EtOH/CH_2Cl_2/NH_4OH$, 1:10:0.1). The product-containing fractions were pooled, evaporated in vacuo, and recrystallized from absolute EtOH to give 1.0 g of analytically pure 11 as its hemihydrate (42%): mp 155 °C (lit.[11] mp 151 °C); ^1H NMR (Me_2SO-d_6) δ 8.26 and 8.16 (s, 2, C_2 H and C_8 H), 7.0 (m, 2, NH_2), 6.00 (d, 1, J = 5 Hz, $C_{1'}$ H), 5.2 (m, 2, OH), 4.7 and 4.3 [2 m, 3 (1:2), $C_{2'}$ H, $C_{3'}$ H, and $C_{4'}$ H), 3.0–2.5 (m, 6, N-CH_2 and CH_2CN), 2.40 (s, 3, CH_3); ^{13}C NMR (D_2O/CH_3OH) 155.9, 153.1, 149.1, 140.6, 89.0, 81.7, 73.6, 72.4, 58.6, 52.6, 41.6, 14.7 ppm; IR (KBr) 2040 (CN), 1660 and 1605 cm^{-1}; UV (H_2O) λ_{max} 258 nm (ϵ 14 920); EIMS, m/e (relative intensity) 334 (M + 1, 3), 281 (5), 237 (3), 194 (40), 180 (30), 178 (10), 136 (100), 135 (60); uniform on electrophoresis. Anal. ($C_{14}H_{20}N_7O_{3.5}$) C, H, N, H_2O.

Attempted Hydroboration of 11. Compound 11 was reacted with a 1 M solution of BH_3 in THF under conditions previously described,[11] as well as by employing twice and four times the amount of BH_3 reported necessary for the transformation of 11 to 13. After workup of the reaction mixtures essentially in the manner described,[11] the obtained material had the following physical characteristics: ^1H NMR (D_2O) δ 8.40 (s, 2), 6.2 (d, 1, J = 5 Hz), 4.9 (t, 1, J = 5 Hz), 4.6 (m, 2), 3.6–4.0 (m, 4), 3.1 (t, 2, J = 6 Hz), 3.05 (s, 3); two spots completely separated, corresponding in their electrophoretic behavior to 11 and 13. The relative amounts of 11 and 13 in the reaction mixtures obtained were determined by analytical HPLC. [Linear gradient elution in 15 min from 100% 0.1 N NH_4OAc/HCO_2H (pH 5.18) to 100% of a 4:1 mixture of 0.1 N NH_4OAc/HCO_2H and MeOH (pH 5.24)]. In our hands, the hydroboration of 11 gave, at best, a 1:10 mixture of 13 and 11.

5′-[(3-Aminopropyl)methylamino]-5′-deoxyadenosine Dihydrochloride (13). Compound 13 was prepared as described for 8 from 5′-chloro-5′-deoxyadenosine[20] (12; 9.0 g, 0.032 mol) and 3-(aminopropyl)methylamine (55 g, 0.625 mol). HPLC indicated two compounds which were completely separated on electrophoresis under the conditions described above. The two compounds were isolated separately by preparative HPLC ($CH_3CO_2NH_4/HCO_2H$ eluant, 5×10^{-2} M, pH 5.3, T_R = 12.5 and 16.8 min). The HPLC fractions containing the second compound were pooled and concentrated in vacuo to yield an oil. This material was purified further by means of an ion-exchange resin column (Dowex WX4 50, NH_4^+) and then by recrystallization of the hydrochloride salt, as already described for 8, to give 13·2HCl: yield 5.3 g (41%); mp 244 °C dec; ^1H NMR (D_2O) δ 8.55 (s, 2, C_2 H and C_8 H), 6.25 (d, 1, J = 5 Hz), 4.8 and 4.6 (2 m, 3, $C_{2'}$ H, $C_{3'}$ H, and $C_{4'}$ H), 3.8 (m, 2, $C_{5'}$ H), 3.6–3.2 (m, 4, N-CH_2), 3.10 (s, 3, CH_3), 2.2 (m, 2, $C_{2''}$ H); ^{13}C NMR (D_2O) 152.7, 150.5, 148.1, 145.6, 121.3, 92.0, 80.9, 75.5, 74.0, 59.9, 55.8, 42.9, 39.2, 24.1 ppm; IR (KBr) 3300 (NH), 1680, 1600 cm^{-1}; UV (H_2O) λ_{max} 258.5 nm (ϵ 15 130); EIMS, m/e (relative intensity) 338 (M + 1, 1), 293 (1), 250 (1), 194 (10), 184 (20), 178 (10), 153 (40), 136 (45), 135 (100), 108 (50); uniform on electrophoresis. Anal. ($C_{14}H_{25}Cl_2N_7O_3$) C, H, Cl; N: calcd, 23.90; found, 23.17.

5′-[[3-(Methylamino)propyl]amino]-5′-deoxyadenosine Trihydrochloride Dihydrate (14). The first eluted compound from the preparative HPLC separation, described for the synthesis of compound 13, was further purified as described for 8 by an ion-exchange resin column and recrystallization of the hydrochloride salt to give 14·3HCl·2H_2O: yield 3.1 g (24%); mp 240 °C dec; ^1H NMR (D_2O) δ 8.50 (s, 2, C_2 H and C_8 H), 6.20 (d, 1, J = 4 Hz, $C_{1'}$ H), 4.8 and 4.5 (2 m, 3, $C_{2'}$ H, $C_{3'}$ H, and $C_{4'}$ H), 3.7 (m, 2, $C_{5'}$ H), 3.5–3.1 (m, 4, N-CH_2), 2.80 (s, 3, CH_3), 2.0–2.5 (m, 2, $C_{2''}$ H); ^{13}C NMR (D_2O) 152.7, 150.6, 147.9, 145.8, 121.1, 92.1, 82.2, 75.8, 73.8, 51.7, 48.7, 47.4, 35.5, 24.9 ppm; IR (KBr) 3400 (NH), 1675 cm^{-1}; UV (H_2O) λ_{max} 258.5 nm (ϵ 14 419); EIMS, m/e (relative intensity) 338 (M + 1, 1), 279 (2), 194 (10), 178 (20), 164 (15), 136 (50), 135 (100); uniform on electrophoresis. Anal. ($C_{14}H_{30}Cl_3N_7O_4$) H, N, Cl, H_2O; C: calcd, 34.83; found, 34.28.

5′-[(Aminoiminomethyl)amino]-2′,3′-(1-methylethylidene)-5′-deoxyadenosine (16). To a suspension of 4 (2.0 g, 6.5 mmol) in 1 N aqueous NaOH (5.2 mL) was added (ethylthio)uronium bromide[27] (2.4 g, 0.013 mol). The pH of the solution was adjusted to 10.5 with 1 N NaOH and maintained for 4 days. The reaction mixture was then neutralized to pH 7 with 1 N HCl and applied to an ion-exchange resin (Dowex WX4 50, NH_4^+). The column was washed with 2 L of water, 0.5 N, 1 N, 2 N, and concentrated NH_4OH (1 L each), and then with 1 N NaOH (300 mL). The latter fraction was neutralized with concentrated HCl to pH 9 and evaporated to dryness. The residue was triturated with MeOH and filtered, and the filtrate was concentrated in vacuo to afford a white solid, which was treated with $CHCl_3$/EtOH absolute [50 mL (45:5)]. This mixture was filtered again, and the filtrate was concentrated in vacuo to obtain a white residue, which was dissolved in *i*-PrOH and reprecipitated by the addition of diethyl ether. These manipulations yielded 1.1 g of 16 (49%): mp 182–184 °C; ^1H NMR (D_2O) δ 8.23 and 8.20 (2 s, 2, C_2 H and C_8 H), 6.20 (d, 1, J = 3 Hz, $C_{1'}$ H), 5.57 (d of d, 1, J = 6 and 3 Hz, $C_{2'}$ H), 5.17 (d of d, 1, J = 6 and 4 Hz, $C_{3'}$ H), 4.50 (m, 1, $C_{4'}$ H), 3.6 (m, 2, $C_{5'}$ H), 1.70 and 1.33 (2 s, 6, CH_3); DCIMS, m/e (relative intensity) 351 (M + 3, 2), 350 (M + 2, 15), 349 (M + 1, 100), 332 (M + 1 − NH_3, 2), 309 (4), 307 (M + 1 − H_2NCN, 6), 213 (4), 136 (adenine, 65); homogeneous on electrophoresis under the conditions described above; no analysis. Used without further purification in the synthesis of 17.

5′-[(Aminoiminomethyl)amino]-5′-deoxyadenosine Dihydrogen Sulfate (17). A solution of 16 (0.52 g, 1.5 mmol) in 1 N aqueous H_2SO_4 (15 mL) was kept at room temperature for 48 h. Addition of absolute EtOH (70 mL) afforded a viscous precipitate, which was dissolved in water (3 mL) and precipitated again with absolute EtOH (30 mL). Repeating this procedure four times afforded 17·H_2SO_4 as a white powdery solid: yield 0.4 g (66%); mp 191 °C (lit.[26] mp of 18·HCl, 150–170 °C dec); ^1H NMR (D_2O) δ 8.10 (s, 2, C_2 H and C_8 H), 6.07 (d, 1, J = 4 Hz, $C_{1'}$ H), 4.9 (d of d, 1, J = 6 and 4 Hz, $C_{2'}$ H), 4.53 (t, 1, J = 6 Hz, $C_{3'}$ H), 4.3 (m, 1, $C_{4'}$ H), 3.67 (d, 2, J = 5 Hz, $C_{5'}$ H); IR (KBr) 3350, 3280 (NH, NH_2), 1680 and 1630 (guanidino) cm^{-1}; UV (H_2O) λ_{max} 258 nm (ϵ 13 280); DCIMS m/e (relative intensity) 309 (M + 1, 1), 136 (adenine, 100); homogeneous on electrophoresis. Anal. ($C_{11}H_{18}N_8O_7S$) C, H, N.

Enzyme Preparation. SAM-DC was prepared from the liver of rats which had been injected with MGBG·2HCl (80 mg/kg of body weight) 22 h before sacrifice according to Pegg.[38] The purification steps were steps 1 and 2 [10500*g* centrifugation and $(NH_4)_2SO_4$ precipitation] used by this author. The specific activity of this preparation was 0.2 nmol of CO_2 min^{-1} (mg of protein)$^{-1}$ when measured under the conditions of Pegg and Williams-Ashman.[28a]

Effects of Enzymatic Activity of Preincubation with Inhibitors or Inhibitors and Sodium Cyanoborohydride. Essentially the conditions described by Pankaskie and Abdel-Monem for these particular experiments[11] were used by us.

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(38) A. E. Pegg, *Biochem. J.*, 141, 581 (1974).