Analogues of S-Adenosylhomocysteine as Potential Inhibitors of Biological Transmethylation. Synthesis of Analogues with Modifications at the 5'-Thioether Linkage

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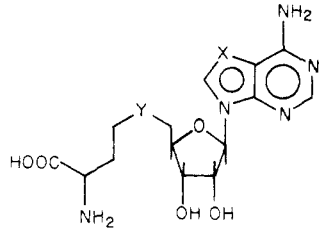
The synthesis of S-adenosylhomocysteine analogues, in which the 5'-thioether linkage is replaced by an oxygen or nitrogen isostere, has been investigated. These compounds were designed to be resistant to enzyme-catalyzed hydrolytic cleavage of the 5'-substituent. The amine analogue **1d** and two amide analogues **20** were prepared via alkylation or acylation of appropriately blocked adenosine derivatives. These new analogues were evaluated as inhibitors of catechol O-methyltransferase and tRNA methylases and found to have poor inhibitory activity.

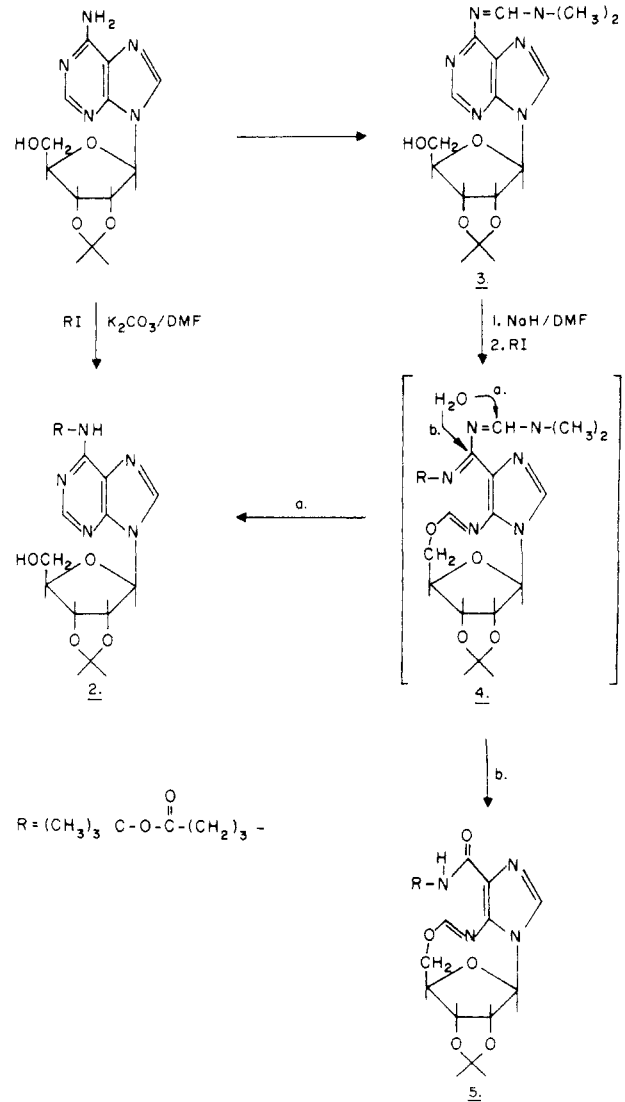
Since S-adenosyl-L-homocysteine (L-SAH, **1a**) is a potent product inhibitor of nearly all S-adenosyl-L-methionine (SAM) dependent methyltransferases investigated,[1] analogues of SAH with structural modifications on the homocysteinyl,[2-4] base,[5,6] and sugar[7-9] moieties have been extensively examined. However, only very limited modification has been made at the thioether linkage, i.e., by oxidation to the sulfoxide or sulfone.[3,4] SAH has been suggested by various investigators to function physiologically via regulation of SAM-dependent methylases. The uptake of SAH is rapid in microorganisms, but fast intracellular degradation by a nucleosidase prevents its accumulation and precludes regulatory function.[10] In search of physiologically stable analogues of SAH, we have synthesized S-tubercidinylhomocysteine (STH, **1b**) which has a stable ribose–purine bond and retains the inhibitory potency of SAH on catechol O-methyltransferase (COMT), indoleethylamine N-methyltransferase, and tRNA methylases.[6] Although the uptake of SAH and its analogues in eucaryotes has not been extensively studied, SAH appears to be metabolized via S-adenosylhomocysteine hydrolase (E.C. 3.3.1.1)[11] yielding adenosine and L-homocysteine as suggested by Walker and Duerre.[12] Their proposal is consistent with our observations on the effects of SAH and STH on stimulated rat lymphocytes in culture.[13] These findings prompted us to attempt the synthesis of SAH analogues which would have a stable linkage between the adenosyl and the α-aminobutyrate moieties.

We chose to synthesize **1c** or **1d** with an isosteric oxygen or nitrogen atom in place of the 5'-sulfur atom of SAH, since enzymic cleavage of an ether or secondary amine is rare. The approach to such compounds by coupling of 5'-tosylate nucleoside with a "hard" nucleophile, such as alkoxide or amine, is not the method of choice because of the competing intramolecular 3,5'-cyclization,[14] although some simple 5'-amino-5'-deoxy nucleosides have been reported by such condensation.[15] In this paper are reported the results of studies of O- and N-alkylation of adenosine derivatives and the total synthesis of 5'-N^{γ}-adenosyl-α,γ-diaminobutyric acid (**1d**).



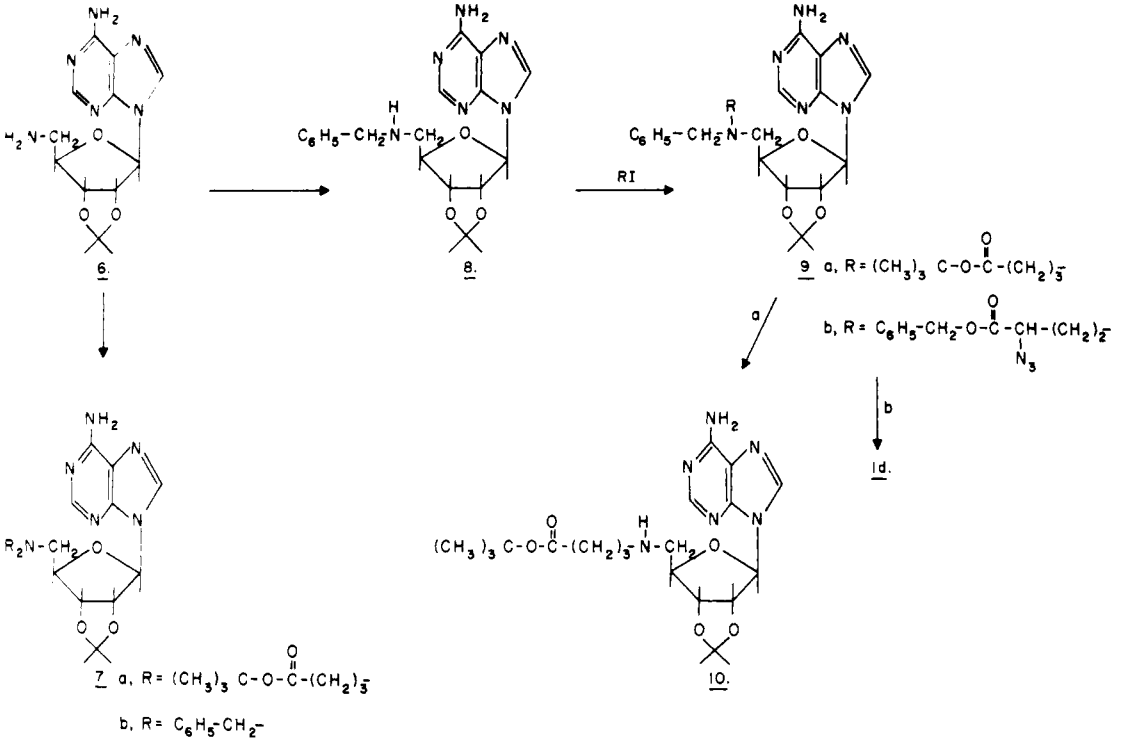
1a, X = N; Y = S (SAH)
b, X = CH; Y = S (STH)
c, X = N; Y = O
d, X = N; Y = NH

Scheme I



Chemistry. 5'-O- vs. N^6-Alkylation. In a model reaction, alkylation of 2',3'-O-isopropylideneadenosine with *tert*-butyl γ-iodobutyrate led to N^6-[(*tert*-butoxycarbonyl)propyl]-2',3'-O-isopropylideneadenosine (**2**, Scheme I) rather than the 5'-O-alkyl derivative, based on the NMR and uv spectra. The chemical shift of the γ-methylene protons at δ 3.65 is upfield of the usual O-methylene group, and a multiplet at δ 6.58 with integration of one proton is indicative of a monoalkylated N^6-amino group. The assignment of N^6-alkylation is consistent with the uv absorption at 268 nm. An attempt to alkylate **3**,

Scheme II



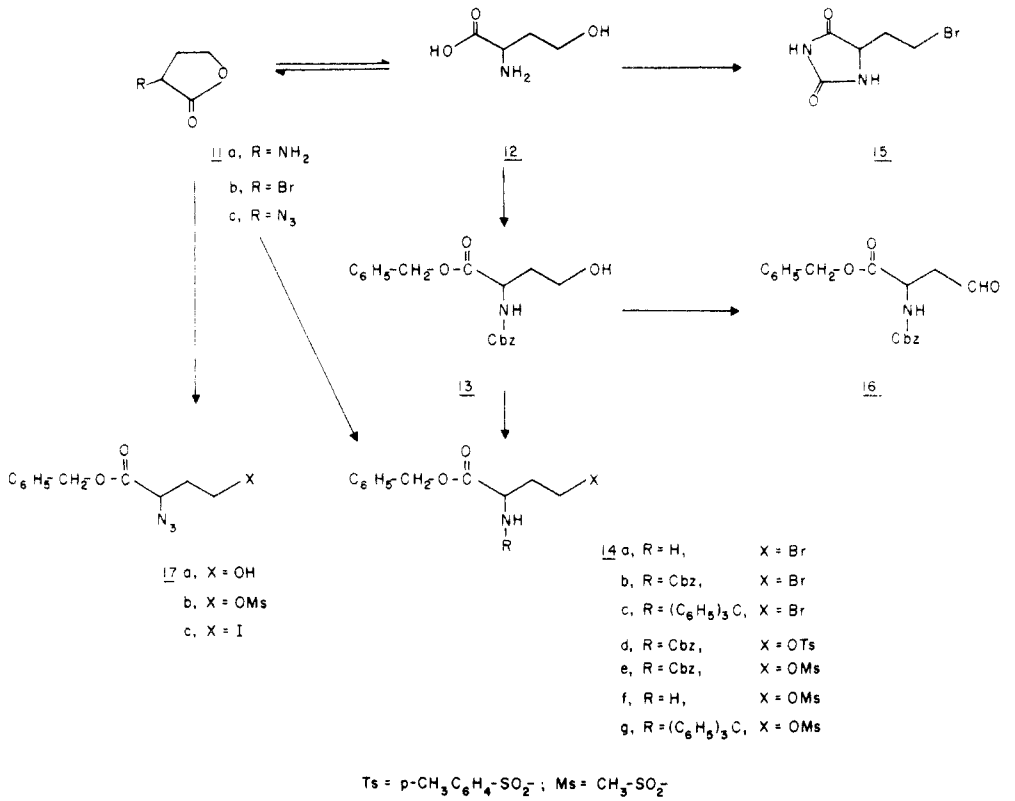
in which the exocyclic N^6-amino group is blocked, resulted in isolation of a nonpurine product, in addition to **2**. NMR spectra indicated loss of the protecting dimethylaminomethylene group in the nonpurine product, since it had an acidic proton at δ 10.5 in addition to a downfield shift of the peaks attributed to H_2 and H_8; both NMR and elemental analysis data suggested that N^6 was replaced by an oxygen. A 1-alkylinosine derivative is consistent with these data, but the uv spectra with λ_{max} at 277 and 288 nm (sh) suggest a conjugated imidazole derivative analogous to urocanic acid[16] or the imidazole derivatives arising from cleavage of a purine nucleoside (e.g., intermediates of histidine biosynthesis[17] and compound IV in ref 14). A plausible structural assignment for this new compound (**5**) is based on a base-catalyzed purine ring-opening mechanism.[18] The pyrimidine ring of 1-alkyladenines opens very easily in weakly basic medium via an imidazole intermediate to undergo $N^1 \rightarrow N^6$ (Dimroth) rearrangement. However, if such a rearrangement is impossible, as when N^6 is blocked, the imidazole derivative **4** might be formed in anhydrous medium (DMF). The hydrolytic decomposition of intermediate **4** during workup could occur via two paths. Attack of water at C-6 (path b) would give amide **5**; alternatively, removal of the N^6-protecting group (path a) would result in regeneration of the free N^6-amino group, which would allow Dimroth rearrangement to **2** as shown in Scheme I.

Mono- vs. Di-N-alkylation. Upon treatment with *tert*-butyl γ-iodobutyrate in DMF in the presence of anhydrous potassium carbonate, 5'-amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (**6**)[19] was converted to **7a** by dialkylation (Scheme II). The monoalkylated derivative **10** was synthesized starting from 5'-benzylamino-5'-deoxyadenosine (**8**), which was prepared by reductive coupling of **6** with benzaldehyde. The use of sodium borohydride in the reduction of the Schiff base formed in situ led to a mixture of **8** and the dibenzylamine derivative **7b**. Apparently, the reductive alkylation did not stop at the secondary amine but proceeded further to the tertiary amine. An alternative procedure using catalytic hydrogenation to reduce the intermediate Schiff base gave a high yield of pure **8**. Alkylation of **8** with *tert*-butyl γ-iodobutyrate gave **9a**. The splittings of the methylene protons of the benzyl group of **9a** to an AB system and a ~0.2 ppm upfield shift of both benzylmethylene and 5'-methylene protons are due to increased steric hindrance to rotation in the tertiary amine. The benzyl group of the tertiary amine was removed by prolonged hydrogenation to obtain the secondary amine **10**.[20]

Alkylating Agents. Scheme III shows the reactions employed in the synthesis of protected α-aminobutyric acid derivatives containing activated γ-alkylating groups which could be condensed to a precursor of **1d**. The γ-bromo compound **14a** was prepared as the ammonium salt by opening of the γ-lactone **11a**[21] in HBr–HOAc, followed by esterification with benzyl alcohol. *N*-Cbz and *N*-trityl compounds **14b** and **14c** were synthesized by conventional procedures.[22] The γ-*O*-sulfonates **14d–g** were synthesized from *N*-carbobenzyloxyhomoserine benzyl ester (**13**) which was prepared from homoserine (**12**) (P. K. Chang, unpublished results). The order of protecting the amino acid functions is important, since the tendency to lactonize is great, via either nucleophilic attack of the carboxylate anion on the γ-bromide or intramolecular dehydration of homoserine itself. Thus, the benzyl group to be used for carboxylic acid protection was introduced in acid medium[23] for the preparation of **14a**, and in basic medium[24] for the preparation of **13**. The exchange of the amino-protecting group from *N*-Cbz of **14e** to *N*-trityl of **14g** was effected through **14f** via a hydrogen bromide deblocking procedure, the γ-mesylate group being able to prevent acid-catalyzed lactonization.

Alkylation of **6** was first attempted with the *N*-Cbz derivatives **14b,d,e**. Under all conditions employed, no coupling was observed; only unreacted starting **6** was detected on TLC. Apparently, the *N*-Cbz group readily undergoes neighboring-group participation as previously reported,[25] leading to decomposition of the reagents. The use of the nonparticipating *N*-trityl group in **14c** and **14g** did not improve the results of alkylation, presumably due

Scheme III



to steric hindrance[22] which inhibits the approach by the 5'-amino group of **6**. In order to avoid both the participation of the carbamate moiety and the steric effect of the trityl group, a bifunctionally protected α-amino acid, **15**,[26] prepared from homoserine, was employed. But the attempted alkylations still failed, probably owing to the instability of the hydantoin in basic media.[27] An alternative preparation of secondary amines is via reductive alkylation.[28] *N*-Carbobenzyloxyaspartic-β-semialdehyde α-benzyl ester (**16**) was synthesized from **13** by a DCC–Me_2SO oxidation.[29] In the reductive alkylation, sodium borohydride was used instead of catalytic methods because of the sensitive Cbz and benzyl ester groups.[30] No condensation with **6** could be observed, even in the presence of various dehydrating agents to drive the formation of the Schiff base.[31,32] The failure of this reductive alkylation seems due to the unfavorable equilibrium of aliphatic imine formation.

In view of the failures described above using conventional amino-protecting groups in the alkylation, we decided to use the azide moiety as a precursor for the amino group of α-aminobutyrate, based on its general stability and ease of reduction. α-Azidobutyric acid γ-lactone (**11c**) was prepared by displacement of the α-bromo lactone (**11b**) with sodium azide. The azido lactone was readily opened to the γ-hydroxybutyrate sodium salt in methanolic sodium hydroxide, as indicated by TLC and disappearance of the lactone ir absorption at 1780 cm^{-1}. Without isolation, the carboxylate was alkylated with benzyl bromide to the ester **17a** and the γ-hydroxy group was converted to the methanesulfonate **17b**. The mesylate was converted to the corresponding γ-iodide **17c**, which was the alkylating agent of choice. The NMR spectra of **17a–c** are in agreement with those of structurally related compounds **13–15**.

Nitrogen Analogue of SAH. Based on the results of model reactions with *tert*-butyl butyrate derivatives, we synthesized *N*-benzyl-*N*-(2',3'-*O*-isopropylideneadenos-5'-yl)-α-azido-γ-aminobutyric acid benzyl ester (**9b**) by alkylation of **8** with **17c**. Compound **9b** was purified by column chromatography on silica gel to give an amphorous solid foam. The presence of the azido and benzyl ester groups on the butyric acid chain was indicated by the characteristic ir absorption at 2100 and 1740 cm^{-1}, respectively. As in compound **9a**, the NMR spectrum shows splitting of the *N*-benzylic protons and an upfield shift of the 5'-methylene indicative of the formation of a 5'-tertiary amine; all chemical shift values are similar to **9a** with variation only due to the change of the butyric acid side chain. Removal of the benzyl groups, conversion of the α-azido to an amino group, and removal of the 2',3'-*O*-isopropylidene group from **9b** were accomplished in one step with palladium–carbon catalyzed hydrogenation in 50% aqueous formic acid. As in the preparation of **10**, complete N-debenzylation required prolonged time and increased hydrogen pressure. Crude 5'-N^{γ}-adenosyl-α,γ-diaminobutyric acid (**1d**) was crystallized from alcohol and desalted on a Dowex 50 cation-exchange column.

In order to gain more insight into the binding mechanism of SAH analogues on methylases, 5'-(β-L-asparaginyl)-5'-deoxyadenosine (**20a**) and 5'-(γ-L-glutaminyl)-5'-deoxyadenosine (**20b**), in which an amide linkage replaced the thioether of SAH, were synthesized. The 5'-aminoacyl nucleosides are expected to have considerable stability as indicated by Robins et al.,[33] and the asparaginyl and glutaminyl chains incorporate the necessary points of attachment of the terminal α-amino acid moiety to the methylases. Appropriately blocked asparatic acid and glutamic acid were coupled to the 5'-amino group of **6** by the active ester method[33] as shown in Scheme IV. Deblocking of **19** by catalytic hydrogenation in 50% aqueous formic acid was effective in removing the isopropylidene, benzyl, and Cbz groups simultaneously as in the synthesis of **1d**.

Biological Results and Discussion. Table I shows the inhibition of partially purified catechol *O*-methyl-

Scheme IV

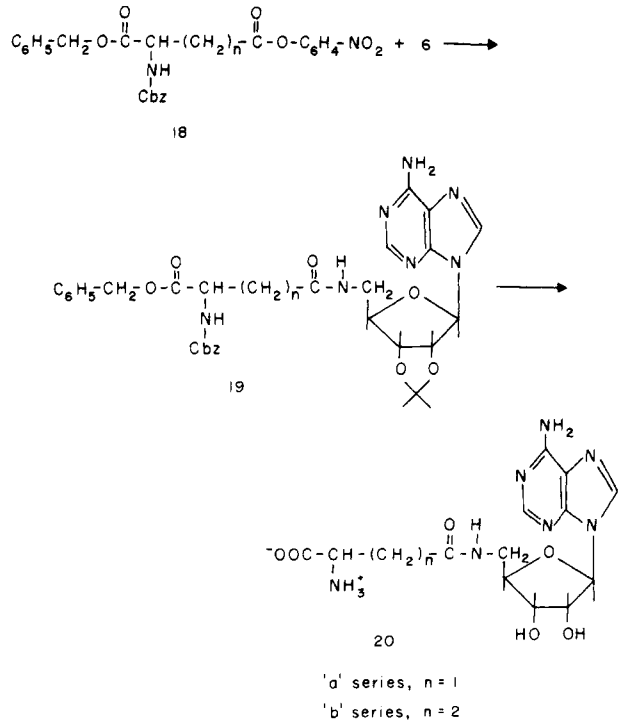


Table I

Compound	Inhibitor concn, mM	% inhibition COMT[a]	% inhibition tRNA methylases[b]
SAH (1a)	0.1	24	77
	0.2	33	83
	0.6	64	
N-Ado-DABA (1d)	0.1	0	20
	0.6	12	
	1.0		61
	1.2	20	
Asp-Ado (20a)	0.1	0	0
	0.6	12	
	1.0		15
	1.2	20	
Glu-Ado (20b)	0.1	0	0
	0.6	11	
	1.0		38
	1.2	19	

[a] Assay conditions:[6] [SAM] = 1.0 mM (0.2 μCi); [dihydroxybenzoic acid] = 2.0 mM; [MgCl] = 1.2 mM; 0.1 M Tris buffer, pH 7.9. [b] Assay conditions:[6] [SAM] = 0.1 mM (0.4 μCi); tRNA = 40 μg; 0.2 M NH_4OAc; 0.1 M Tris buffer, pH 8.6.

transferase (COMT) and tRNA methyltransferases (tRNA MT) by the structural analogues of SAH which have an amine or amide substituted for the thioether linkage. These nitrogen analogues showed substantial reduction in inhibitory activity on these two enzymes as compared with SAH. **1d** inhibited COMT and tRNA MT to the extent of 20% at concentrations of 1.2 and 0.1 mM, respectively. A linear competitive pattern of inhibition was observed in the inhibition of tRNA MT by **1d**, and a K_i of 350 μM was obtained from the Dixon plot shown in Figure 1. Thus, **1d** has only ca. $^1/_{15}$ the inhibitory potency of SAH (K_i = 25 μM).[10]

The results presented herein employing nitrogen analogues of SAH lead to a conclusion similar to that of Borchardt et al.[4] that the sulfur atom is important for optimal binding to these highly specific methylases. The

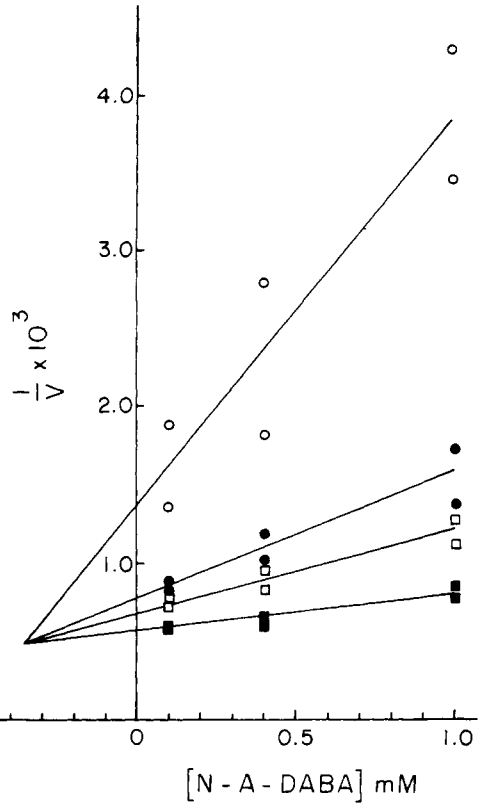


Figure 1. Inhibition of tRNA MT by **1d** (*N*-Ado-DABA) at SAM concentrations of 10 (○), 25 (●), 50 (□), and 100 μM (■). The assay conditions are given in Table I, footnote *b*.

similar magnitude of inhibition by the amine **1d** and the more bulky amides **20a** and **20b** suggests that the decreased affinity of these analogues is not due to a simple steric effect but is probably due to loss of an intramolecular sulfur–purine interaction and/or a combination of electronic effects. The well-known phenomenon of inversion of the Cotton effect from negative to positive, as a result of the substitution of a sulfur atom at the 5'-carbon of adenosine, has been attributed to either conformational factors[34] or electronic interaction.[35] Our NMR and microanalytical data indicated the blocked and deblocked adenosine derivatives with a 5'-amino substituent were more tightly solvated with both ethyl acetate and water than were the 5'-sulfur adenosine derivatives. Evidently the 5'-amino compounds have considerably different dipole moment and hydrogen-bonding interactions compared to the corresponding sulfur compounds. Whether the conformation of **1d** differs significantly from SAH when bound to the methylases is still an open question.

Although the secondary amine **1d** was more effective on tRNA MT than the aminoacyl analogues **20a** and **20b**, none of the nitrogen analogues provided inhibition as effective as SAH against either COMT or tRNA MT. For this reason, no evaluation of these compounds with SAH hydrolase was pursued in the present investigation.

Experimental Section[36]

All melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer. NMR spectra were determined on a Varian T-60A spectrometer using tetramethylsilane as an internal standard. Elemental microanalyses were carried out by Baron Consulting Co., Orange, Conn. Analytical thin-layer chromatography (TLC) was carried out on precoated Eastman (6060) silica gel sheets and preparative thick-layer chromatography (PLC) on precoated Brinkman (5766) 2-mm plates. Aprotic organic extracts were routinely dried over

anhydrous magnesium sulfate and filtered through Celite (Fisher C-211). Solvents were then removed under reduced pressure on a rotary evaporator. 2',3'-*O*-Isopropylideneadenosine and DL-homoserine were obtained from Sigma. 5'-Amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (**6**),[19] 5-(β-bromoethyl)hydantoin (**15**),[26] *N*-carbobenzyloxy-α-benzyl-β-(*p*-nitrophenyl)-L-aspartate (**18a**),[37] and *N*-carbobenzyloxy-α-benzyl-γ-(*p*-nitrophenyl)-L-glutamate (**18b**)[38] were prepared by literature procedures and had the expected melting points and spectral characteristics.

***tert*-Butyl γ-Iodobutyrate.** γ-Chlorobutyric acid (10 ml, ~0.1 mol) was added to a mixture of 25 ml (~0.5 mol) of isobutene (condensed in 20 ml of dry ether) and concentrated H_2SO_4 (2 ml) in a pressure bottle at 0°. After sealing the bottle, the suspension was stirred at room temperature overnight, after which the ether phase was removed from the chilled bottle and washed throughly with water and saturated $NaHCO_3$ solution. The dried ether extract was evaporated in vacuo to give analytically pure γ-chlorobutyric acid *tert*-butyl ester as a colorless liquid: 10.2 g (57%); ir (neat) 1725 cm^{-1} (C=O, ester); NMR ($CDCl_3$) δ 1.43 (s, 9, *tert*-butyl), 2.10 (m, 2, β-CH_2), 2.38 (m, 2, α-CH_2), 3.56 (t, 2, γ-CH_2). Anal. ($C_8H_{15}ClO_2$) C, H, Cl.

The chloro ester prepared above (0.8 g, 5 mmol) was refluxed overnight in 25 ml of methyl ethyl ketone saturated with NaI (~10%). The suspension was filtered and the filtrate was evaporated to dryness. The residue was triturated with ether and the ether extract was evaporated to give a light yellow liquid. The NMR spectrum revealed this product as a mixture of iodo and chloro derivatives in the ratio of 4 to 1: NMR ($CDCl_3$) δ 3.22 (γ-CH_2).

N^6-(*tert*-Butoxycarbonylpropyl)-2',3'-*O*-isopropylideneadenosine (2). Isopropylideneadenosine (300 mg, 1 mmol) and 35 mg (~1.5 mmol) of NaH were added in that order to 5 ml of dry DMF under N_2 at 0°. The solution was stirred at room temperature for ~30 min until evolution of H_2 had ceased. Crude γ-iodobutyric acid *tert*-butyl ester (400 mg) was then injected into the solution, and the reaction was heated at 60 °C for 2 h. After cooling, the solution was poured into ice–water and extracted with $CHCl_3$ (2 × 30 ml). The $CHCl_3$ extract was washed thoroughly with water, dried, and evaporated to an oil which showed four components on TLC. The oily residue was chromatographed on PLC and the major component with R_f 0.48 was eluted (EtOAc–hexane, 3:1): ir (neat) 1720 (C=O), 1620 cm^{-1} (C=N); NMR ($CDCl_3$) δ 1.43 (s, 9, *tert*-butyl), 2.0 (m, 2, β-CH_2), 2.33 (dd, 2, α-CH_2), 3.65 (dd, 2, γ-CH_2), 3.9 (m, 2, $H_{5'}$), 5.90 (d, 1, $H_{1'}$, $J_{1',2'}$ = 3.5 Hz), 6.58 (br m, 1, 6-NH), 7.80 (br s, 1, H_2), 8.28 (s, 1, H_8); uv λ_{max} (EtOH) 268 nm (ε 16.8 × 10^3). Anal. ($C_{21}H_{31}N_5O_6$) C, H, N.

N^6-[(Dimethylamino)methylene]-2',3'-*O*-isopropylideneadenosine (3). 2',3'-*O*-Isopropylideneadenosine (1.1 g, 3.6 mmol) was dissolved in dry *N*,*N*-dimethylformamide (DMF) (20 ml) and treated with 1.5 ml of *N*,*N*-dimethylformamide diethyl acetal. The resulting solution was stirred at room temperature for 24 h as described previously[39] and evaporated to dryness (30–40 °C, 1 mm). The residue was codistilled with toluene to remove DMF until a crystalline product was obtained. Recrystallization of the solid from $CHCl_3$–petroleum ether gave pure white crystals: 1.15 g (89%); mp 170–171 °C; NMR ($CDCl_3$) δ 3.20 (d, 6, *N*,*N*-dimethyl), 4.5 (m, 1, $H_{4'}$), 5.16 (m, 2, $H_{2'}$ and $H_{3'}$), 5.90 (d, 1, $H_{1'}$, $J_{1',2'}$ = 4 Hz), 7.90 (br s, 1, H_2), 8.48 (s, 1, H_8). Anal. ($C_{16}H_{22}N_6O_4$) C, H, N.

5'-*O*,N^5-Cycloimido-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-*N*-(*tert*-butoxycarbonylpropyl)carboxamide (5). N^6-(Dimethylaminomethylene)-2',3'-*O*-isopropylideneadenosine (**3**, 360 mg, 1 mmol) was alkylated with *tert*-butyl γ-iodobutyrate via the same procedure as described for **2**. The resultant oily residue from the reaction mixture, which showed two major spots on TLC, was chromatographed on PLC; one major band, eluted and characterized by TLC and NMR, was identical with **2**. A second major band was eluted with R_f 0.70 (EtOAc–hexane, 3:1): ir (neat) 1720 (C=O, ester), 1695 (C=O, conj amide), 1575 cm^{-1} (C=N); NMR ($CDCl_3$) δ 3.91 (m, 2, $H_{5'}$), 4.54 (t, 2, γ-CH_2), 6.02 (d, 1, $H_{1'}$, $J_{1',2'}$ = 4 Hz), 8.10 (s, 1, H_2), 8.66 (s, 1, H of cycloimidate), 10.5 (s, 1, H of carboxamide); uv (EtOH) λ_{max} 277 nm (ε 21.0 × 10^3), shoulder 288 (14.7 × 10^3); λ_{max} (0.1 N OH^-) 268 nm (ε 24.0 × 10^3); λ_{max} (0.1 N H^+) 277 and 288 nm. Anal. ($C_{21}H_{30}N_4O_7$) C, H, N.

5'-[*N*,*N*-Di(*tert*-butoxycarbonylpropyl)]amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (7a). The 5'-aminoadenosine derivative **6** (200 mg, 0.65 mmol) was stirred with anhydrous K_2CO_3 (100 mg, 0.7 mmol) in dry DMF (3 ml) at 70–80 °C under nitrogen, while 260 mg (~1 mmol) of crude *tert*-butyl γ-iodobutyrate was added via syringe in 50-mg portions at 30-min intervals. After addition was complete, the reaction was continued for an additional 3 h until no appreciable starting amine **6** was observed on TLC. After removal of DMF under high vacuum, the oily residue was partitioned between $CHCl_3$ (2 × 30 ml) and water. The organic extract was washed thoroughly with water, dried, and evaporated to give a light yellow oil, which was dissolved in EtOAc and chromatographed on PLC. The major uv-absorbing band corresponding to the alkylated amino nucleoside was separated, the product extracted with EtOAc, and the extract evaporated to give a glassy product that was homogenous on TLC: R_f 0.31 (EtOAc–hexane, 3:1); yield 150 mg (38%); ir (neat) 1725 (C=O, ester), 1630 cm^{-1} (C=N); NMR ($CDCl_3$) δ 1.42 [s, 18, *tert*-butyl, overlapped $^1/_2C(CH_3)_2$], 1.65 [m, 4, β-CH_2, overlapped $^1/_2C(CH_3)_2$], 2.16 (br d, 4, α-CH_2), 2.41 (br d, 4, γ-CH_2), 2.70 (dd, 2, $H_{5'}$), 4.33 (td, 1, $H_{4'}$), 5.0 (dd, 1, $H_{3'}$, $J_{3',4'}$ = 4 Hz, $J_{2',3'}$ = 7 Hz), 5.50 (dd, 1, $H_{2'}$), 6.06 (d, 1, $H_{1'}$, $J_{1',2'}$ = 2.5 Hz), 6.28 (br s, 2, 6-NH_2), 7.92 (s, 1, H_2), 8.33 (s, 1, H_8); uv λ_{max} (EtOH) 259 nm (ε 13.5 × 10^3). Anal. ($C_{29}H_{46}N_6O_7$·dipicrate) C, H, N.

5'-Benzylamino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (8). The amine **6** (910 mg, 3 mmol) was mixed with 1.5 g (14 mmol) of acid free benzaldehyde in ethanol (15 ml), to which 10% Pd/C (300 mg) was added, and hydrogenated under 30 psi at room temperature overnight. The catalyst was removed by filtration and washed with ethanol, and the filtrate was evaporated to a colorless oil which was shown to be a mixture of **8** and benzyl alcohol. TsOH (600 mg, 3.1 mmol) in EtOAc (5 ml) was used to precipitate the amino nucleoside as a toluenesulfonate salt, which was triturated with dry ether (20 ml). The gummy residue was triturated with ether (ice bath) to give a white powder, which was then collected by filtration and rinsed with ether. This very hydroscopic salt was dissolved in absolute methanol (10 ml), to which 360 mg (3.2 mmol) of potassium *tert*-butoxide was added. Potassium tosylate was precipitated by trituration with ether (30 ml) and removed by filtration. The collected salt was washed with $CHCl_3$, and the combined filtrate was evaporated to dryness. The residue was partitioned between chloroform and saturated $NaHCO_3$ solution; the $CHCl_3$ extract was washed again with $NaHCO_3$ solution, then dried ($MgSO_4$), and evaporated to a colorless oil. After drying at high vacuum, a white solid foam was obtained which was homogenous on TLC: R_f 0.08 (EtOAc–hexane, 3:1); 1.1 g (2.8 mmol, 93% yield); NMR ($CDCl_3$) δ 2.32 (br s, collapsed with D_2O, 1, NH), 2.92 (d, 2, $H_{5'}$), 3.80 (s, 2, CH_2 of benzylamine), 4.42 (m, 1, $H_{4'}$), 5.08 (dd, 1, $H_{3'}$, $J_{2',3'}$ = 7 Hz, $J_{3',4'}$ = 3.5 Hz), 5.50 (dd, 1, $H_{2'}$, $J_{1',2'}$ = 3.5 Hz), 6.02 (d, 1, $H_{1'}$), 6.50 (br s, 2, 6-NH_2), 7.22 (s, 5, ArH), 7.83 (s, 1, H_2), 8.10 (s, 1, H_8); uv λ_{max} (EtOH) 259 nm (ε 18.5 × 10^3). Anal. ($C_{20}H_{24}N_6O_3$·dipicrate·$3H_2O$) C, H, N.

5'-(*N*,*N*-Dibenzyl)amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (7b). The 5'-aminoadenosine derivative **6** (610 mg, 2 mmol) was dissolved in a solution of dry methanol (10 ml), benzaldehyde (1 ml, 10 mmol), and acetic acid (0.5 ml). The resulting solution was stirred at room temperature for 1 h and then chilled in an ice bath, and 800 mg (21 mmol) of $NaBH_4$ was added in 50-mg portions. The reaction mixture was maintained at pH 7 by occassional addition of acetic acid. After gas evolution had ceased, the reaction was brought to room temperature and stirred for an additional 1 h. Methanol was removed by evaporation and the residue was partitioned between $CHCl_3$ (30 ml) and water. Additional $CHCl_3$ (15 ml) was used to extract the water layer. The combined organic extract was washed twice with water, dried, and evaporated to a colorless oil. The residue was triturated with a solution of *p*-toluenesulfonic acid (380 mg, 2 mmol) in EtOAc and free amine was generated with 225 mg (2 mmol) of potassium *tert*-butoxide as described for **8**. The crude free amine was shown to be a mixture of **8** (minor), **7b** (major), and other impurities by TLC and NMR spectra. An analytical sample of **7b** was prepared by PLC and was obtained as a homogenous solid foam: R_f 0.38 (EtOAc–hexane, 3:1); NMR ($CDCl_3$) δ 2.70 (d, 2, $H_{5'}$), 3.60 (d, 4, CH_2 of benzylamine) 4.38 (m, 1, $H_{4'}$), 4.82 (dd, 1, $H_{3'}$, $J_{2',3'}$ = 7 Hz, $J_{3',4'}$ = 3.5 Hz), 5.26 (dd, 1, $H_{2'}$), 6.0 (d, 1, $H_{1'}$,

$J_{1',2'}$ = 2.5 Hz), 6.15 (br s, 2, 6-NH_2), 7.22 (s, 10, ArH), 7.70 (s, 1, H_2), 8.08 (s, 1, H_8); uv λ_{max} (EtOH) 259 nm (ϵ 15.7 × 10^3). Anal. ($C_{27}H_{30}N_6O_3$) C, H, N.

tert-**Butyl** *N*-**Benzyl**-*N*-**(2',3'-***O*-**isopropylideneadenos-5'-yl)-γ-aminobutyrate (9a).** Crude **8** (200 mg, 0.5 mmol) was alkylated with γ-iodobutyric acid *tert*-butyl ester by the procedure described for **7a**. The oil obtained from the reaction mixture was purified by addition of toluenesulfonic acid (190 mg) and the free amine was regenerated with potassium *tert*-butoxide (100 mg) as described for **8**. The crude free amine was further purified by PLC eluted with EtOAc to obtain a glassy oil (130 mg, 48%): homogeneous on TLC; R_f 0.31 (EtOAc–hexane, 3:1); ir (neat) 1720 (C=O, ester), 1635 cm^{-1} (C=N); NMR ($CDCl_3$) δ 1.40 [s, 9, *tert*-butyl, overlapping with $^1/_2C(CH_3)_2$], 1.85 (t, 2, β-CH_2), 2.18 (d, 2, α-CH_2), 2.45 (d, 2, γ-CH_2), 2.70 (dd, 2, H_5), 3.60 (q, 2, CH_2 of *N*-benzyl, J_{gem} = 14 Hz), 6.02 (d, 1, $H_{1'}$, $J_{1',2'}$ = 2 Hz), 7.22 (m, 5, benzyl aromatic), 7.80 (s, 1, H_2), 8.20 (s, 1, H_8); uv λ_{max} (EtOH) 259 nm (ϵ 17.2 × 10^3). Anal. ($C_{28}H_{38}N_6O_5$·dipicrate) C, H, N.

tert-**Butyl** *N*-**(2',3'-***O*-**Isopropylideneadenos-5'-yl)-γ-aminobutyrate (10).** The fully blocked amine **9a** (85 mg, 0.16 mmol) was dissolved in 95% ethanol (5 ml) containing 10% Pd/C (50 mg). The suspension was hydrogenated under 40 psi at room temperature for 2 days until no appreciable starting material, **9a**, could be detected by TLC. After removal of the catalyst by filtration, and evaporation of the filtrate to dryness, the residue still appeared to have a trace of **9a** by TLC and NMR. Therefore an analytical sample of **10** was purified by PLC and eluted with EtOAc: yield 25 mg (0.056 mmol, 35% yield); NMR ($CDCl_3$) δ 1.40 (s, 9, *tert*-butyl), 1.80 (m, 2, β-CH_2), 2.23 (m, 2, α-CH_2), 2.62 (m, 2, γ-CH_2), 2.85 (d, 2, H_5), 3.10 (br s, 1 collapsed with D_2O, NH), 7.92 (s, 1, H_2), 8.32 (s, 1, H_8); the resonances attributed to the other sugar protons are identical with those in the benzylaminoadenosine derivative **10**; uv λ_{max} (EtOH) 259 nm (ϵ 13.1 × 10^3). Anal. ($C_{21}H_{32}N_6O_5$·dipicrate) C, H, N.

Benzyl α-Amino-γ-bromobutyrate (14a). α-Amino-γ-bromobutyric acid hydrobromide[21] (1.5 g, 5.7 mmol) was suspended in a mixture of dry benzene (30 ml), benzyl alcohol (30 ml), and *p*-toluenesulfonic acid (1.2 g, 6.3 mmol). The mixture was heated at reflux for 4–5 h during which time water was removed by distillation. The clear solution was cooled and the amine salt crystallized by addition of benzene (20 ml) and an excess of anhydrous ether. The needles were collected and washed with ether. Recrystallization from EtOH–ether gave 2.1 g (83%), mp 147–148 °C.

The amine salt (3.5 g, 7.9 mmol) was stirred in saturated aqueous sodium carbonate solution (150 ml) and extracted with ether (two 100-ml portions). The combined ether extract was washed with water, dried, and evaporated to give analytically pure free amine as a light yellow oil: 1.75 g (6 mmol, 76%); ir (neat) 3300 (NH_2), 1740 cm^{-1} (C=O ester); NMR ($CDCl_3$) δ 1.60 (s, collapsed by D_2O, 2, NH_2), 2.20 (m, 2, β-CH_2), 3.52 (t, 2, γ-CH_2, J = 7.5 Hz), 3.63 (m, 1, α-CH), 5.14 (s, 2), and 7.35 (s, 5, benzyl ester). Anal. ($C_{11}H_{14}NBrO_2$) C, H, N, Br.

Benzyl *N*-Carbobenzyloxy-α-amino-γ-bromobutyrate (14b). The amino acid ester **14a** prepared above (1.2 g, 4.4 mmol) was added to a mixture of ether (20 ml) and H_2O (20 ml) containing $NaHCO_3$ (870 mg, 10 mmol) in an ice bath. Carbobenzyloxy chloride (CbzCl, 1 ml, ~6 mmol) was added slowly with stirring, and the mixture was then brought to room temperature and stirred for an additional 1 h. The ether layer was separated, dried, and evaporated to dryness. Trituration with petroleum ether yielded 1.5 g of crystalline product. Recrystallization from ether–petroleum ether gave analytically pure white needles: mp 60–65 °C; ir (KBr) 3280 (NH), 1685 cm^{-1} (*N*-Cbz); NMR ($CDCl_3$) δ 4.60 (m, 1, α-CH), 5.40 (br d, 1, NH), 5.15 (s, 2, CH_2 of *N*-Cbz), 5.20 (s, 2, CH_2 of benzyl ester). Anal. ($C_{19}H_{20}NBrO_4$) C, H, N, Br.

Benzyl *N*-Trityl-α-amino-γ-bromobutyrate (14c). The free amine **14a** (560 mg, 2.1 mmol) and triethylamine (0.4 ml, ~4 mmol) were stirred in dry CH_2Cl_2 (55 ml) at 0° following which 550 mg (2 mmol) of trityl chloride was added. The solution was stirred for 3 h as Et_3N·HCl precipitated. The mixture was poured into ice–water and extracted with $CHCl_3$. The extract was washed twice with water, dried, and evaporated to a light yellow oil which was essentially homogeneous on TLC (ninhydrin negative, becoming positive after 3 days). An analytical sample was purified by PLC: NMR ($CDCl_3$) δ 2.80 (br d, 1, NH), 3.40 (m, 3, α-CH and γ-CH_2), 4.56 (q, 2, CH_2 of benzyl ester, J_{gem} = 12 Hz), 7.2–7.5 (m, 15, trityl and benzyl aromatic). Anal. ($C_{30}H_{28}BrO_2$) C, H, N, Br.

Benzyl *N*-Carbobenzyloxy-α-amino-γ-hydroxybutyrate (13) (*N*-Carbobenzyloxyhomoserine Benzyl Ester). DL-Homoserine (**12**, 2.38 g, 20 mmol) and 2.1 g (20 mmol) of Na_2CO_3 were added to H_2O (20 ml) in an ice bath, following which 3.5 ml (20 mmol) of CbzCl was added. The suspension was then brought to room temperature over 4 h, until no obvious ninhydrin reaction was observed in extracts from the reaction solution. The solution was lyophilized and residual water codistilled with toluene (three 10-ml portions) to give a semisolid. This residue was suspended in dry DMF (15 ml) on an ice bath, and 3 ml (~22 mmol) of benzyl bromide was added. The mixture was kept at room temperature for 1–2 days; after evaporation to dryness, the residue was partitioned between EtOAc and water. The organic extract was dried, evaporated to an oil, and then triturated with petroleum ether; crystallization was effected by scratching or seeding at 0°. Recrystallization from ether and petroleum ether gave an analytically pure white crystalline material: 2.0 g (5.8 mmol, 29% from homoserine); mp 60–61 °C; ir (KBr) 3500 (OH), 3300 (NH), 1750 (C=O ester), 1680 cm^{-1} (*N*-Cbz); NMR ($CDCl_3$) δ 2.0 (m, 2, β-CH_2), 2.45 (br m, collapsed with D_2O, 1, OH), 3.65 (q, 2, γ-CH_2), 4.60 (m, 1, α-CH). The benzyl ester and *N*-Cbz protons are observed at the same chemical shift as in **14b**. Anal. ($C_{19}H_{21}NO_5$) C, H, N.

Benzyl *N*-Carbobenzyloxy-α-amino-γ-*O*-(toluenesulfonyl)butyrate (14d). To 690 mg (2 mmol) of **13** dissolved in 10 ml of dry pyridine at 0° was added 630 mg (3.3 mmol) of *p*-tolylsulfonyl chloride. The solution was kept in the refrigerator for 2 days, then poured into ice–water, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with chilled 3 N HCl, water, saturated $NaHCO_3$, and again with water. The dried organic layer was evaporated and the oily residue was again partitioned between $CHCl_3$ and water; the $CHCl_3$ extract was dried, decolorized with active carbon, and evaporated to an essentially pure, colorless oil: 840 mg (84%). An analytical sample was obtained by PLC: ir (neat) 1360 cm^{-1} (–SO_2–); NMR ($CDCl_3$) δ 2.36 (s, 3, $ArCH_3$), 4.04 (t, 2, γ-CH_2, J = 6.5 Hz), 7.2 and 7.7 (q, 4, ArH). Anal. ($C_{26}H_{27}NSO_7$) C, H, N, S.

Benzyl *N*-Carbobenzyloxy-α-amino-γ-*O*-(methanesulfonyl)butyrate (14e). To 1 g (3 mmol) of **13** dissolved in dry pyridine (10 ml) at 0 °C was added methanesulfonyl chloride (0.5 ml, ~5 mmol). The reaction was worked up after 2 days as described for the tosylate derivative **14d**. The resulting oily residue was crystallized from ether and petroleum ether to give 0.95 g (86%) of white prisms: mp 65–66 °C. An analytical sample was obtained by a second recrystallization from ether and petroleum ether: ir (KBr) 1360 cm^{-1} (–SO_2–); NMR ($CDCl_3$) δ 2.82 (s, 3, mesyl), 4.2 (t, 2, γ-CH_2, J = 6.5 Hz). Anal. ($C_{18}H_{23}NSO_7$) C, H, N, S.

Benzyl *N*-Trityl-α-amino-γ-*O*-(methanesulfonyl)butyrate (14g). The *N*-carbobenzyloxyamine **14e** (1 g, 2.6 mmol) was suspended in glacial acetic acid (2 ml), to which 30–32% HBr–HOAc (2 ml) was added. The solution was stirred for 5–6 min and when gas evolution ceased, dry ether (50 ml) was added to precipitate the hydrobromide salt. The gummy salt was dissolved in chloroform, then precipitated by, and washed with ether. The ether-insoluble residue was redissolved in chloroform (50 ml) and washed with saturated $NaHCO_3$ solution (2 × 50 ml). The $CHCl_3$ extract was dried to give free amine, **14f**, as a colorless oil which was homogeneous (ninhydrin positive) on TLC: NMR ($CDCl_3$) δ 2.3 (br s, collapsed with D_2O, 2, NH_2), 4.4 (m, 3, overlapped γ-CH_2 and α-CH).

This free amine (**14f**, 750 mg, 3 mmol) was tritylated with the same procedure described for **14c**. The resulting oil had TLC and chemical characteristics similar to **14c**: NMR ($CDCl_3$) δ 3.52 (dd, 1, α-CH), 4.56 (q, 2, CH_2 of benzyl ester, J_{gem} = 12 Hz). Anal. ($C_{31}H_{31}NSO_5$) C, H, N.

Attempted Condensation of 5'-Amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (6) with Alkylating Agents. To 3 ml of dry DMF or HMPT was added 0.5 mmol of **6** and anhydrous K_2CO_3 (0.5 mmol), and the resulting mixture was heated under nitrogen. Various alkylating agents (**15**, **14a–c**, 1–5 mmol) were injected and the course of the reactions was followed by TLC.

Using reaction temperatures from 70 °C to 110 °C and reaction times up to 12 h, the 5'-aminoadenosine derivative **6** was recovered essentially unchanged.

***N*-Carbobenzyloxyaspartic-β-semialdehyde α-Benzyl Ester (16).** *N*-Carbobenzyloxyhomoserine benzyl ester **13** (340 mg, 1 mmol) was dissolved in 5 ml of dry Me_2SO containing 618 mg (3 mmol) of dicyclohexylcarbodiimide (DCC), 50 mg (0.7 mmol) of dry pyridine, and anhydrous H_3PO_4 (65 mg, 0.5 mmol). The mixture was stirred at room temperature overnight, then poured into ice–water, and extracted with ether. The ether extract was washed with water three times, dried, and evaporated to a colorless oil which showed two components on TLC. Pure **16** was obtained by PLC: NMR ($CDCl_3$) δ 3.0 (d, 2, β-CH_2, $J_{\alpha,\beta}$ = 5 Hz), 4.65 (d, t, 1, α-CH, $J_{N,\alpha}$ = 8 Hz, $J_{\alpha,\beta}$ = 5 Hz), 9.60 [s, 1, –C(=O)H]. Anal. ($C_{19}H_{19}NO_5$) C, H, N.

A crystalline dinitrophenylhydrazone derivative of **16** was prepared by stirring an ether solution of crude **16** with dinitrophenylhydrazine in diglyme at room temperature to give a brown oil precipitate. The oily precipitate was extracted with EtOAc and washed with H_2O, $NaHCO_3$, and again with H_2O, dried, and evaporated. Crystallization from EtOAc–ether in an ice bath gave an orange solid: 400 mg (77% from **14**); mp 115–116 °C; NMR ($CDCl_3$) δ 3.0 (t, 2, β-CH_2), 7.4–9.1 (m, 3, ArH), 10.90 (br s, 1, HNN). Anal. ($C_{25}H_{23}N_5O_8$) C, H, N.

Attempted Reductive Alkylation of 5-Amino-5'-deoxy-2',3'-*O*-isopropylidineadenosine (6) with *N*-Carbobenzyloxyaspartic-β-semialdehyde α-Benzyl Ester (16). The amino nucleoside **6** (0.5 mmol) and excess **16** were stirred in dry THF (5 ml) with and without acid (CH_3COOH, CF_3COOH, TsOH) catalysis. The reactions were followed by TLC; no indication of imine formation was observed at ambient or elevated temperature. Attempts to force the condensation reaction by use of desiccants ($MgSO_4$, Na_2SO_4, molecular sieves) and a reducing agent ($NaBH_4$) all failed to yield the desired alkylated amine.

α-Azidobutyric Acid γ-Lactone (11c). α-Bromobutyric acid γ-lactone (**11b**, 16 g, 100 mmol) (Aldrich) was stirred with 13 g (200 mmol) of NaN_3 in Me_2SO (100 ml) at room temperature for 30 min and then 60 °C for 30 min. After cooling, the brown mass was poured into ice–water and extracted with $CHCl_3$ (2 × 100 ml). The combined $CHCl_3$ extract was washed thoroughly with water and filtered through a pad of active carbon and anhydrous $MgSO_4$. The filtrate was evaporated to a homogenous yellow oil: 9.0 g (71%); ir (neat) 2100 (N_3), 1780 cm^{-1} (lactone); NMR ($CDCl_3$) δ 2.4 (m, 2, β-CH_2), 4.3 (m, 3, α-CH and γ-CH_2).

Benzyl α-Azido-γ-hydroxybutyrate (17a). The azido lactone prepared above (7.0 g, 55 mmol) was added slowly to a 10% methanolic NaOH solution (25 ml, 60 mmol). The exothermic reaction was carried out at room temperature for 1 h, at which time the reaction appeared to be complete by TLC and ir data. $NaHCO_3$ (400 mg, 4.7 mmol) was added to neutralize the excess NaOH, and all solvent was then removed by evaporation and codistillation of residual H_2O with toluene. The semisolid residue was suspended in dry DMF (100 ml), benzyl bromide (10 ml, 60 mmol) was added, and the mixture was stirred at room temperature for 2 days. After evaporation of DMF in vacuo, the residue was partitioned between $CHCl_3$ (2 × 100 ml) and ice–water. The $CHCl_3$ extract was washed three to four times with ice–water, decolorized, dried, and evaporated to give a light yellow oil (12 g, quantitative). The crude product had R_f 0.44 (EtOAc–hexane, 3:1) with trace amounts of impurities. An analytical sample was prepared by PLC: ir (neat) 3300 (OH), 2100 (N_3), 1735 cm^{-1} (C=O, ester); NMR ($CDCl_3$) δ 2.0 (m, 2, β-CH_2), 2.3 (br s, collapsed with D_2O, 1, OH), 3.66 (t, 2, γ-CH_2, $J_{\beta,\gamma}$ = 6 Hz), 4.13 (dd, 1, α-CH), 5.20 (s, 2, CH_2 of benzyl ester). The hydroxy ester thus prepared was unstable and tended to cyclize to the γ-lactone above room temperature. Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

Benzyl α-Azido-γ-*O*-(methanesulfonyl)butyrate (17b). Crude **17a** (10 g, 42 mmol) was dissolved in cool, dry pyridine (100 ml) in an ice bath, and 5.7 g (50 mmol) of mesyl chloride was added, stirred for 1 h, and kept in the refrigerator overnight. The brown mixture was poured into cracked ice and extracted with $CHCl_3$ (2 × 100 ml), and the $CHCl_3$ extract was washed with chilled H_2O, 3 N HCl, H_2O, saturated $NaHCO_3$, and H_2O. The organic layer was decolorized, dried, and evaporated to an orange oil (15 g) which showed only minor impurities by TLC and NMR. A 10-g portion of the oily residue was dissolved in ether (50 ml) and applied to a silica gel column (400 g, 25 × 5 cm). The column was eluted with 1:1 ether–petroleum ether (300 ml), followed by ether (500 ml) at a flow rate of 10 ml/min, with 50-ml fractions taken. Fractions 10–14 gave 6.5 g (75%) of essentially pure mesylate after evaporation of solvent. An analytical sample was obtained by PLC of a small portion of the product eluted from the column. Evaporation of the PLC extract gave a homogeneous colorless oil: R_f 0.30 (EtOAc–hexane, 1:3); ir (neat) 2100 (N_3), 1740 (C=O, ester), 1350 cm^{-1} ($-SO_2-$); NMR ($CDCl_3$) δ 2.93 (s, 3, CH_3), 4.26 (t, partially overlapping with α-CH, 2, γCH_2, $J_{3,\gamma}$ = 5.5 Hz). Anal. ($C_{12}H_{15}N_3SO_5$) C, H, N, S.

Benzyl α-Azido-γ-iodobutyrate (17c). Mesylate **17b** obtained from the silica gel column as described above (6.2 g, 20 mmol) was refluxed in 10% NaI in methyl ethyl ketone (15 ml, 100 mmol) for 2 h, after which the mixture was evaporated to dryness. The residue was suspended in ether (50 ml); the salts were filtered off and washed with additional ether. The combined ether extract was treated with active carbon, filtered, and evaporated to give a yellow oil which was homogeneous on TLC: yield 7.0 g (quantitative); NMR ($CDCl_3$) δ 3.20 (t, 2, γ-CH_2, $J_{\beta,\gamma}$ = 6.5 Hz). Anal. ($C_{11}H_{12}N_3IO_2$) C, H, N, I.

Benzyl *N*-Benzyl-*N*-(2',3'-*O*-isopropylideneadenos-5'-yl)-α-azido-γ-aminobutyrate (9b). 5'-Benzylamino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (**8**, 800 mg, 2 mmol) was stirred with 420 mg (3 mmol) of anhydrous K_2CO_3 in dry DMF (10 ml) under nitrogen and was alkylated with 1 g (2.9 mmol) of benzyl α-azido-γ-iodobutyrate (**17c**) by the method described for the synthesis of **7**. The reaction mixture was worked up by the procedure described for **9a** to give a crystalline toluenesulfonate salt, which was then dissolved in dry methanol (20 ml). To regenerate the free amine, Na_2CO_3 (210 mg, 2 mmol) was substituted for potassium *tert*-butoxide. The suspension was stirred at room temperature for 1 h and excess anhydrous ether was added. The salt was removed by filtration and washed with $CHCl_3$, and the combined filtrate was evaporated to a solid foam. The crude product was dissolved in EtOAc and put on a silica gel column (80 g, 30 × 2.5 cm). The column was eluted (flow rate 2 ml/min, 5-ml fractions) with 1 l. of EtOAc. Fractions 30–90 containing **9b** were combined and evaporated to give a solid foam which was homogeneous on TLC: R_f 0.35 (EtOAc–hexane, 3:1); yield 310 mg (25%); ir (KBr) 2100 (N_3), 1740 (C=O, ester), 1640 cm^{-1} (adenyl); NMR ($CDCl_3$) δ 2.0 (m, 2, $-CH_2$), 2.7 (m, 4, γ-CH_2 and $H_{5'}$), 3.6 (m, 2, CH_2 of *N*-benzyl), 4.22 (m, 1, α-CH), 4.36 (m, 1, $H_{4'}$), 4.95 (dd, 1, $H_{3'}$, $J_{2',3'}$ = 7 Hz, $J_{3',4'}$ = 3.5 Hz), 5.22 (d, 2, CH_2 of benzyl ester), 5.42 (dd, 1, $H_{2'}$, $J_{1',2'}$ = 2 Hz), 6.02 (d, 1, $H_{1'}$), 6.45 (br s, 2, 6-NH_2), 7.22 (m, 5, *N*-benzylic aromatic), 7.30 (s, 5, phenyl of benzyl ester), 7.88 (s, 1, H_2), 8.18 (s, 1, H_8); uv λ_{max} (EtOH) 259 nm (ε 14.5 × 10^3). Anal. ($C_{31}H_{35}N_9O_5$·dipicrate·$2H_2O$) C, H, N.

5'-*N*$^{\gamma}$-Adenosyl-α,γ-diaminobutyric Acid (1d). To 250 mg (0.4 mmol) of **9b** suspended in 50% aqueous formic acid (20 ml) was added 150 mg of 10% Pd/C. The mixture was hydrogenated at 50 psi at room temperature for ca. 24 h. The catalyst was removed by filtration and washed with water. The combined filtrate was lyophilized and residual H_2O removed by two azeotropic distillations with methanol. The glassy residue was triturated with ethanol to give a white crystalline product, which was recrystallized from H_2O and absolute EtOH (uv and ninhydrin positive). TLC on silica gel indicated a major spot at R_f 0.47 (pyridine–MeOH–H_2O, 1:5:20). The light tan crystalline material (120 mg) was desalted by ion-exchange chromatography (Dowex 50-X8, H^+ form, 250 × 10 mm). Salts were eluted with H_2O (100 ml), and **1d** was then eluted with 0.2 M H_2CO_3–Et_3N buffer (400 ml, pH gradient 4–11.9) at a flow rate of 2.5 ml/min (5-ml fractions). The effluent was monitored by uv (254 nm), and fractions 56–71 with R_f 0.47 were combined and lyophilized to give 100 mg (66%) of a white powder. TLC indicated the product was still contaminated with a trace uv and ninhydrin-positive spot at R_f 0.65 which disappeared after a second hydrogenolysis: mp 215–220 °C dec; uv λ_{max} (H_2O) 259 nm (ε 17.0 × 10^3); NMR (D_2O) δ 5.95 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.5 Hz), 8.06 (s, 1, H_2 or H_8), 8.15 (s, 1, H_2 or H_8). Anal. ($C_{14}H_{21}N_7O_5$·3HCOOH) C, H, N.

5'-(*N*-Carbobenzyloxy-α-benzyl-β-L-asparaginyl)-5'-deoxy-2',3'-*O*-isopropylideneadenosine (19a). To dry DMF (5 ml) containing Et_3N (0.15 ml, 1.4 mmol) was added 305 mg (1

mmol) of **6** and 480 mg (1 mmol) of *N*-carbobenzyloxy-α-benzyl-β-(*p*-nitrophenyl)-L-aspartate (**18a**).[37] The yellow solution was stirred at room temperature for 2 h, when TLC showed that the reaction was complete. DMF was evaporated in vacuo to give an oily residue which was dissolved in EtOAc and washed with 1 N NaOH and water. The organic extract was dried, evaporated to a small volume, and triturated with ether to give a crystalline product. Recrystallization from EtOAc–ether gave 450 mg (0.71 mmol, 71% yield) of analytically pure amide (**19a**): mp 105–108 °C; ir (KBr) 1710 (C=O ester), 1640 cm^{-1} (amide + C=N); NMR ($CDCl_3$) δ 3.0 (d, 2, β-CH_2, $J_{\alpha,\beta}$ = 5.5 Hz), 3.3 (m, 2, H_5), 4.65–4.75 (m, 2, α-CH overlapping with H_3), 5.76 (d, 1, $H_{1'}$, J = 4.5 Hz), 7.78 (s, 1, H_2), 8.3 (s, 1, H_8). Anal. ($C_{31}H_{35}N_7O_8$) C, H, N.

5'-(β-L-Asparaginyl)-5'-deoxyadenosine (20a). The totally blocked amide **19a** (50 mg, 78 μmol) dissolved in 50% aqueous HCOOH was allowed to stand overnight at room temperature, then 20 mg of 10% Pd/C was added, and the mixture was hydrogenated at 15 psi overnight at room temperature. The catalyst was removed by filtration, the filtrate was lyophilized, and the residue triturated with ethanol to yield a crystalline product. The crude product was redissolved in water and filtered through a millpore filter, then recrystallized from H_2O–ethanol, and washed with EtOH to give an analytical sample: 20 mg (59%); R_f 0.71 (pyridine–MeOH–H_2O) on TLC (uv and ninhydrin positive); mp >240 °C dec; ir (KBr) 3000–2500 (br, $-NH_3^+$), 1630 cm^{-1} (C=N); uv λ_{max} (H_2O) 259 nm (ε 1.5 × 10^4). Anal. ($C_{14}H_{19}N_7O_6 \cdot HCOOH \cdot H_2O$) C, H, N.

5'-(*N*-Carbobenzyloxy-α-benzyl-γ-L-glutaminyl)-5'-deoxy-2',3'-*O*-isopropylideneadenosine (19b). *N*-Carbobenzyloxy-α-benzyl-γ-(*p*-nitrophenyl)-L-glutamate (**18b**,[38] 500 mg, 1 mmol) was added to a stirring solution of 300 mg (1 mmol) of **5** in dry DMF (5 ml) containing 150 μl (1.5 mmol) of triethylamine. The light yellow solution was stirred overnight at room temperature and the product isolated as described for **19a**. It was not possible to crystallize the product, but the amorphous solid foam obtained was essentially pure by TLC: NMR ($CDCl_3$) δ 2.36 (m, 4, β- and γ-CH_2), 3.25 (m, 2, $H_{5'}$), 4.42 (m, 2, α-CH overlapping with $H_{4'}$), 5.82 (d, 1, $H_{1'}$, J = 4.5 Hz). Anal. ($C_{32}H_{37}N_7O_8$) C, H, N.

5'-(γ-L-Glutaminyl)-5'-deoxyadenosine (20b). The totally blocked glutamate derivative **19b** (180 mg, 0.28 mmol) was deblocked by the same procedure as described for the preparation of **20a** to give analytically pure light tan crystals in quantitative yield, 110 mg. The crystals were dried at 100 °C, 0.25 mmHg, for 24 h: mp 190–195 °C dec; uv λ_{max} (H_2O) 259 nm (ε 1.42 × 10^4). Anal. ($C_{15}H_{21}N_7O_6 \cdot 1.5HCOOH$) C, H, N.

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