Synthesis and Evaluation of Analogues of 5′-([(Z)-4-Amino-2-butenyl]methylamino)-5′-deoxyadenosine as Inhibitors of Tumor Cell Growth, Trypanosomal Growth, and HIV-1 Infectivity[†]

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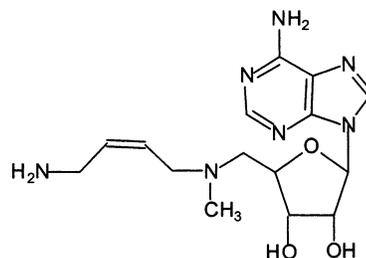
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A well-defined series of 5′-([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxyadenosine analogues was designed and synthesized in order to further ascertain the optimal structural requirements for *S*-adenosylmethionine decarboxylase inhibition and potentially to augment and perhaps separate their antiproliferative and antitrypanosomal activities. Most structural modifications had a deleterious affect on both the antitrypanosomal and antineoplastic activity of 5′-([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxyadenosine. However, di-O-acetylation of the parent compound produced a potential prodrug that caused markedly pronounced inhibition of trypanosomal and neoplastic cell growth and viability. Moreover, the acetylated derivative of 5′-([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxyadenosine did inhibit HIV-1 growth and infectivity, whereas the parent compound did not.

Introduction

S-Adenosylmethionine decarboxylase (AdoMetDC) is a key enzyme in the regulation and synthesis of polyamines.[1] The decarboxylation of *S*-adenosylmethionine (AdoMet) by AdoMetDC represents a potential metabolic switching point between cellular methylation reactions and polyamine biosynthesis.[2] AdoMet can donate a methyl group by acting as a cofactor in a variety of cellular reactions (e.g., DNA methyltransferase).[3,4] Furthermore, if AdoMet is decarboxylated by AdoMetDC, it can donate a propylamine moiety in the synthesis of the polyamines, spermidine, and spermine.[2] Since polyamines play a critical role in both the origin and progression of neoplastic phenotypes and in the life cycle of trypanosomes, inhibition of AdoMetDC by a variety of drugs has led to the development of agents possessing both antineoplastic and antiparasitic activity.[2,3] Of interest to our research group is a prototypical irreversible inhibitor of AdoMetDC, 5′-([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxyadenosine (AbeAdo), originally synthesized by chemists at Merrell Dow Research Institute where it was designated MDL73811 (Figure 1).[5] As illustrated in Figure 2, it has been hypothesized that binding of AbeAdo to AdoMetDC results in the formation of a Schiff base between the butenylamine functionality of AbeAdo and a pyruvate prosthetic group within the enzyme's active site. Subsequent enzyme-

cis-5′-Deoxy-5′-(4-amino-2-butenyl)methylaminoadenosine

Figure 1. Structure of AbeAdo (MDL73811).

mediated abstraction of a proton α to the Schiff base causes the formation of a conjugated imine that is highly susceptible to nucleophilic attack by other residues within the enzyme.[2,5] Despite AbeAdo's high affinity and pronounced inhibition of AdoMetDC in cell-free assays[2,5,6] and its salient in vitro activity against a variety of cancer cell lines, it possessed only moderate growth inhibitory activity when tested in vivo.[2,7,8] Conversely, AbeAdo has shown good efficacy, in vivo, against a variety of trypanosomal strains such as *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*.[2,9,10] The greater efficacy of AbeAdo as an inhibitor of trypanosomal growth, as opposed to mammalian cells, has been attributed to the active uptake of AbeAdo by trypanosomes via a purine transport system not found in mammalian cells.[2,11]

The promising antitrypanosomal and antineoplastic activity of AbeAdo clearly warranted further investigation. Therefore, a well-defined series of AbeAdo analogues was designed and synthesized in order to further ascertain the optimal structural requirements for AdoMetDC inhibition and potentially to augment and perhaps separate their antiproliferative and antitrypanosomal activities.

Figure 2. Proposed mechanism of AbeAdo. This figure was adapted from the following: Casara, P.; Marchal, P.; Wagner, J.; Danzin, C. 5′-{[(*Z*)-4-Amino-2-butenyl]methylamino}-5′-deoxyadenosine: A Potent Enzyme-Activated Irreversible Inhibitor of *S*-Adenosyl-L-methionine Decarboxylase from *Escherichia coli. J. Am. Chem. Soc* **1989**, *111*, 9111−9113.

Scheme 1. Synthesis of Acetylated Analogues of AbeAdo

2, R = OH, R' = OH
3, R = OH, R' = H

4, R = OH, R' = OH
5, R = OH, R' = H

6, R = OH, R' = OH
7, R = OH, R' = H

8, X = Boc, R = OC(O)CH$_3$, R' = OC(O)CH$_3$
9, X = H,   R = OH,   R' = H
**10**, X = H,   R = OC(O)CH$_3$, R' = OC(O)CH$_3$
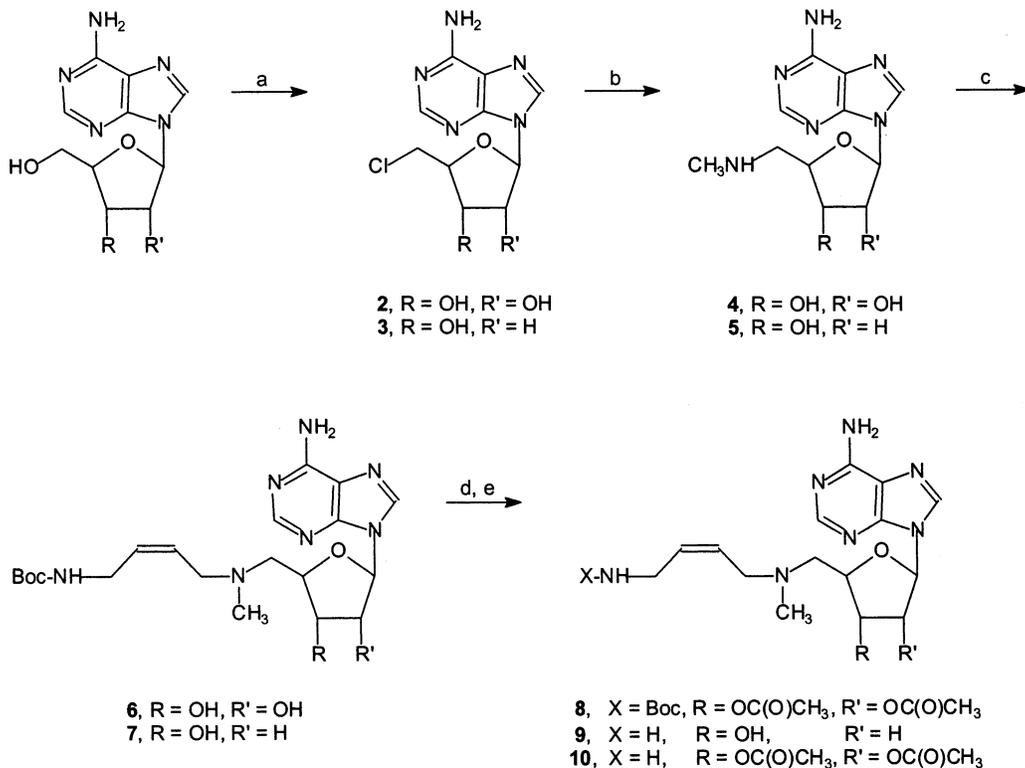
Chemistry

Syntheses of **9**, the 2′-deoxy derivative of AbeAdo and of **10**, the di-O-acetylated derivative of AbeAdo, are shown in Scheme 1. The 5′-hydroxyl group of 2′-deoxyadenosine or adenosine was chlorinated with thionyl chloride according to Robbins et al.[12] The respective halogenated intermediates **2** and **3** were then aminated with anhydrous methylamine to give the corresponding methylamino analogues **4** and **5**, respectively. Compounds **4** and **5** were N-alkylated with *cis-tert*-butoxycarbonyl-4-chloro-2-butenyl-1-amine to yield **6** and **7**.[5] Intermediate **6**, after treatment with trifluoroacetic acid, gave **9**, the desired 2′-deoxy AbeAdo

analogue. Intermediate **7** was O-acetylated and then treated with trifluoroacetic acid for selective removal of the *tert*-butyloxycarbonyl protecting groups to give **10**, the di-O-acetylated analogue of AbeAdo.

As illustrated in Scheme 2, the synthesis of the 2′,3′-seco-adenosine analogues **14** and **16** was accomplished by an oxidative−reductive cleavage of 5′-deoxy-5′-chloroadenosine to give 5′-deoxy-5′-chloro-2′,3′-seco-adenosine, **11**,[13] which was aminated and N-alkylated as previously described[5] to give **13**. Deprotection of **13** yielded analogue **14** directly. When intermediate **13** was O-acetylated and then selectively deprotected, analogue **16** was obtained.

Scheme 2. Synthesis of 2′,3′-Seco Analogues of AbeAdo[a]

2, R = R' = OH   **11**, R = R' = OH

14, R = R' = OH
16, R = R' = OC(O)CH₃
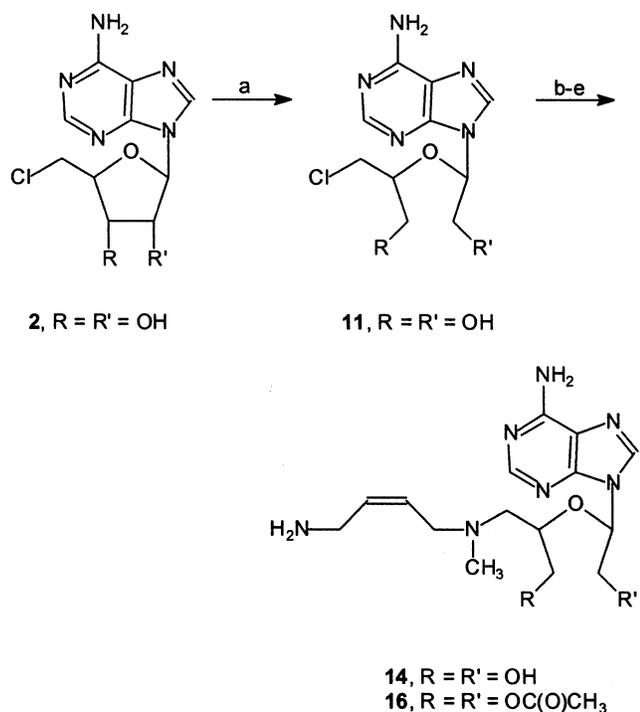
[a] (a) NaIO₄/NaBH₃; (b) methylamine; (c) *cis-tert*-butoxycarbonyl-4-chloro-2-butenyl-1-amine; (d) acetic anhydride/DMAP; (e) trifluoroacetic acid.

Synthesis of the phenyl derivatives of AbeAdo, **24** and **25**, is shown in Scheme 3. The 2′,3′-isopropylidene derivative of adenosine was chlorinated and aminated to give analogues **18** and **19**, respectively. Intermediate **19** was then N-alkylated with either α-bromo-*p*-toluenitrile or α-bromo-*o*-toluenitrile to yield **20** and **21**, respectively. The nitrile groups of **20** and **21** were reduced with LiAlH₄, and the isopropylidene groups were removed under acidic conditions to give the desired analogues **24** and **25**. The 2-aminoadenosine derivative of AbeAdo, **30**, was synthesized in an analogous manner (Scheme 4).

The synthesis of **36**, a ribose isostere of AbeAdo, is illustrated in Scheme 5. Adenine was N-alkylated with acetylethoxymethyl bromide to give **31**. The acetyl group was saponified, and the corresponding alcohol was chlorinated as previously indicated to yield **33**. Amination with methylamine, followed by alkylation with the butenyl arm moiety and final deprotection, was done as previously described to yield the expected final analogue **36**.
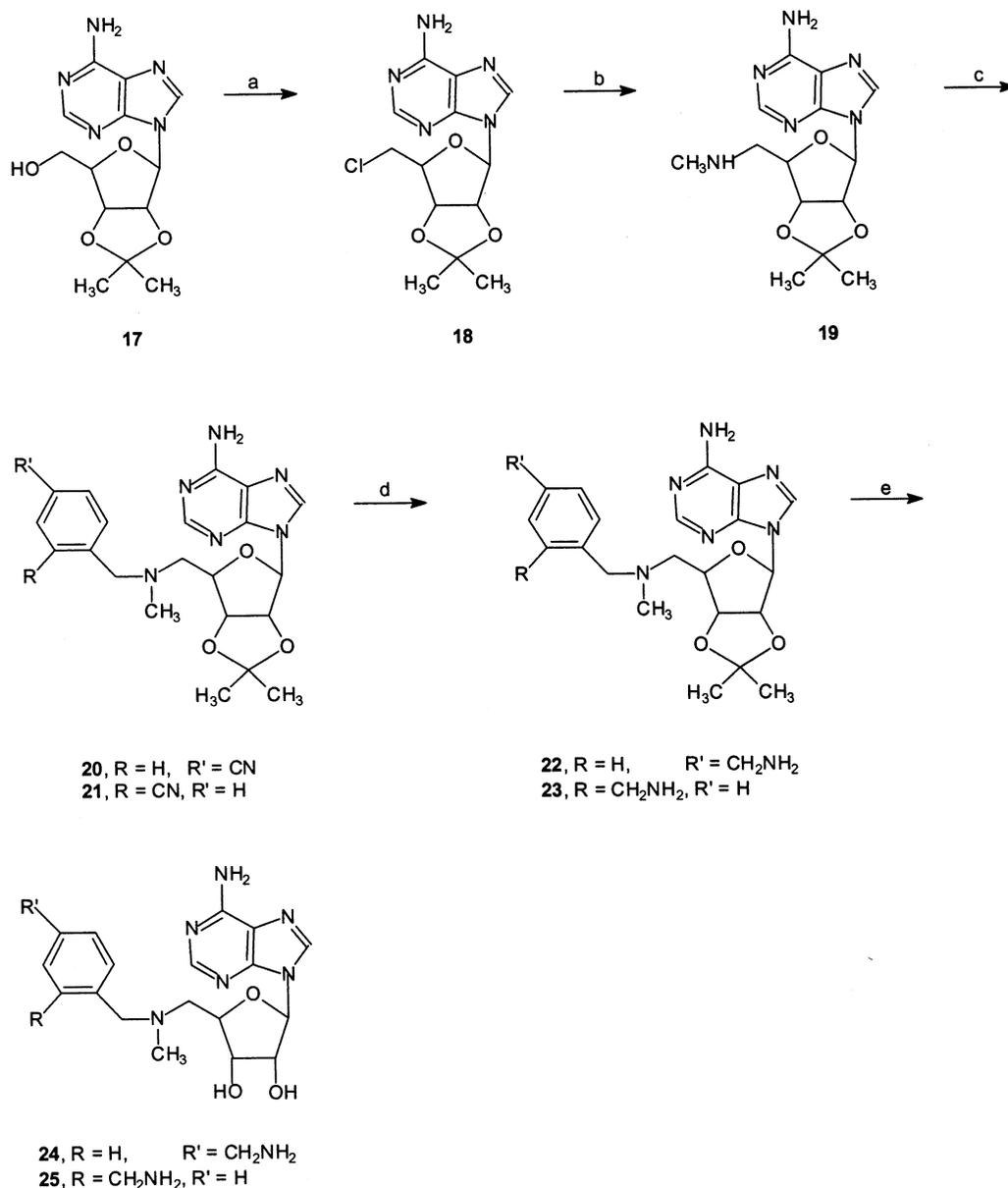
Biological Results and Discussion

In Vitro Antitrypanosomal Activity. The in vitro antitrypanosomal activities of AbeAdo and its related analogues were determined in the LAB 110 EATRO isolate of *Trypanosoma brucei brucei* and three clinical isolates of *Trypanosoma brucei rhodesiense*: KETRI 243 (melarsoprol- and diamidine-resistant), KETRI 269, and KETRI 243 As 10-3 (highly arsenical resistant). The data are shown in Table 1. In this assay, compound **10** displayed the most pronounced inhibition of trypanosomal growth. The remaining analogues, with the exception of **4**, **24**, and **25**, were also growth inhibitory.

Evaluation of the biological data presented in Table 1 offered serveral key insights into the structure−activity relationships (SAR) of these analogues. Unsurprisingly, absence of the butenyl moiety resulted in a loss of activity, as seen with AbeAdo versus analogue **4**. This result supports the previously proposed mechanism of action of AbeAdo, whereby AbeAdo binds to the active site of AdoMetDC and forms a Schiff base with a pyruvate prosthetic group. The enzyme then abstracts a proton from the substrate to give a conjugated imine.[5] The lack of activity displayed by the phenyl derivatives of AbeAdo, **24**, and **25** is consistent with this proposed mechanism. The phenyl substituents of **24** and **25**, which can be viewed as isosteric replacements for the *cis*-butenyl arm of AbeAdo, can potentially form a Schiff base, but not a conjugated imine (which requires proton abstraction) within the enzyme's active site.

Several analogues of AbeAdo, **9**, **14**, and **36**, all containing modifications of the ribose ring, were synthesized. A previous observation that 2′-deoxyadenosine is a substrate of 5′-methylthioadenosine phosphorylase in trypanosomes but not in human tumor cells[14] prompted us to synthesize **9**, the 2′-deoxy derivative of AbeAdo, as a possible means to increase its selectivity toward trypanosomes. In fact, the in vitro antitrypanosomal activity of **9** was ∼40 times lower than that of AbeAdo as seen in the standard EATRO 110 strain of *T. b. brucei* (Table 1). This suggests that the 2′-hydroxyl group of AbeAdo plays an active role in the inhibition of this enzyme. The uptake of 2′-deoxyAbeAdo (not determined) may also be a contributing factor to the reduced activity of **9** compared to AbeAdo. Moreover, when the inhibitory potencies of **9** and AbeAdo toward mammalian AdoMet DC were compared, an even greater differential in activity was observed; compound **9** was >250 times less active than AbeAdo (Table 2). This result indicates that the 2′-hydroxyl group of AbeAdo makes a substantial contribution to inhibition of the mammalian enzyme. The 2′, 3′-seco derivative of AbeAdo, **14**, was synthesized in order to increase the conformational freedom of the ribose ring, yet maintain a structure very similar to that of a ribose moiety. As seen with the 2′-deoxy derivative **9**, the acyclic analogue **14** displayed significantly less activity than the parent compound. Similarly, the antitrypanosomal activity of compound **36**, whose hydroxyethoxymethyl functionality can serve as a ribose ring isostere (e.g., acyclovir), was substantially decreased compared to AbeAdo (Table 1). The findings that compounds **9**, **14**, and **36** display consistently lowered biological activities compared to AbeAdo indicate that the ribose ring of AbeAdo is highly sensitive to structural modifications.

AbeAdo analogue **30**, which contains 2,6-diaminopurine in place of adenine, was synthesized to determine what effects, if any, modifications to the purine substituent might have on the antitrypanosomal activity of AbeAdo. Disappointingly, this modification caused at least an order of magnitude decrease in activity.

Finally, to potentially improve the cellular uptake of these AbeAdo-derived structures, the O-acetylated derivatives of certain analogues were prepared. Acetylation of 2′,3′-seco-AbeAdo, **14**, gave **16**, whose in vitro antitrypanosomal activity showed an increase, on average, of 6-fold in the EATRO and 243 strains, compared

**Scheme 3.** Synthesis of Benzyl Derivatives of AbeAdo[a]

20, R = H, R' = CN
21, R = CN, R' = H

22, R = H,   R' = CH$_2$NH$_2$
23, R = CH$_2$NH$_2$, R' = H

24, R = H,   R' = CH$_2$NH$_2$
25, R = CH$_2$NH$_2$, R' = H

[a] (a) Thionyl chloride; (b) methylamine; (c) α-bromo-*p*-tolunitrile or α-bromo-*o*-tolunitrile; (d) LiAlH$_4$; (e) H$_2$SO$_4$.

Scheme 4. Synthesis of 2-Amino Derivative of AbeAdo[a]

26   **30**
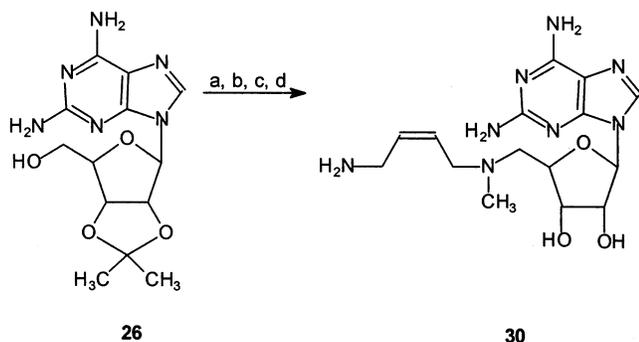
[a] (a) Thionyl chloride; (b) methylamine; (c) *cis-tert*-butoxycarbonyl-4-chloro-2-butenyl-1-amine; (d) H$_2$SO$_4$.

to that of its unacetylated parent compound, **14** (Table 1). Similarly, **10**, the di-O-acetylated derivative of AbeAdo, was 10 times more active in vitro than AbeAdo in the EATRO and 269 strains (Table 1).

In Vivo Antitrypanosomal Activity. AbeAdo and its acetylated derivative, **10**, were further tested in mice against several trypanosomal infections (Table 3). When mice infected with *T. b. brucei* LAB 110 EATRO were treated with **10**, this AbeAdo derivative was curative in several dose regimens. The activity of **10** paralleled that of the parent compound, AbeAdo, when dosed in two equal injections (b.i.d). Compound **10** was also active in the same strain when administered by continuous infusion using 7 day pumps. The di-O-acetylated derivative, **10**, was protective but not curative against *T. b. rhodesiense* (KETRI 243), a strain that is resistant to the arsenical, melarsoprol (Arsobal), and the diamidine pentamidine. This strain was more sensitive to the parent compound. AbeAdo was curative for four out of five mice receiving 100 mg kg^{-1} day^{-1} in 7 day pump dosing. In contrast, compound **10**, at the highest dose administered, increased life span by ~68% (Table 4). Since compound **10** was ~4 times more active in vitro

Scheme 5. Synthesis of Ethoxymethyl Ribose Analogue of AbeAdo[a]
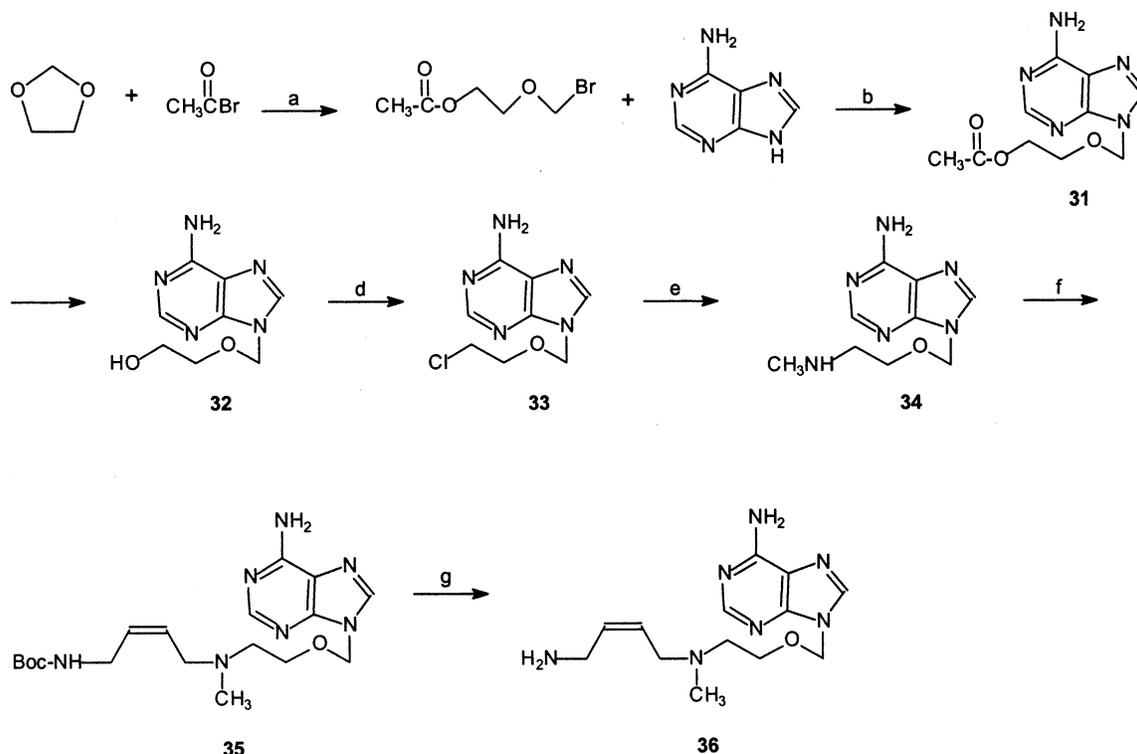
[a] (a) Stir at room temp; (b) NaH; (c) sodium methoxide; (d) thionyl chloride; (e) methylamine; (f) *cis-tert*-butoxycarbonyl-4-chloro-2-butenyl-1-amine; (g) H_2SO_4.

Table 1. In Vitro Antitrypanosomal Activity of AbeAdo Derivatives (IC$_{50}$, μM)[a]

compound	trypanosomal strain			
	110[b]	243[c]	269[c]	243 As 10-3[c]
AbeAdo	0.1	0.04	0.22	0.1
4	44% @ 100 μM	26% @ 100 μM	d	d
9	4.2	2.9	74	30
10	0.014	0.014	0.02	0.054
14	16	15	d	d
16	1.8	4.6	3.8	2.9
24	21% @ 100 μM	23% @ 100 μM	d	d
25	14	42% @ 100 μM	d	d
30	4.5	3.8	19.0	2.0
36	10	6.6	11	0.96

[a] IC$_{50}$ is the concentration required to inhibit the growth of trypanosomes by 50%. [b] EATRO strain. [c] KETRI strain. [d] Not determined.

Table 2. In Vitro Inhibition of AdoMetDC from L1210 Murine Leukemia Cells

compound	ID$_{50}$ (μM)[a]
AbeAdo	0.078
9	20
10	0.82
14	27% inhibition at 100 μM
24	21% inhibition at 100 μM
25	41% inhibition at 100 μM
36	>100

[a] ID$_{50}$ value is the concentration required to inhibit the assay of AdoMetDC activity by 50%.

Table 3. In Vivo Activity of AbeAdo and Its Acetylated Derivative, **10**, against *T. b. brucei* LAB 110 EATRO

compound	daily dose (mg/kg)	treatment time (days)	MSD[a] (days)	(no. cured)/total
control			3	0/5
AbeAdo	10 ip (b.i.d.)	3	12	4/5
	25 ip (b.i.d.)	3	>34	5/5
	50 ip (b.i.d.)	3	>34	5/5
10	10 ip (b.i.d.)	3	17	4/5
	25 ip (b.i.d.)	3	33	4/5
	50 ip (b.i.d.)	3	>34	5/5
	25 (pump)	7	>34	5/5
	50 (pump)	7	>34	5/5
	100 (pump)	7	26	4/5

[a] MSD is the mean survival in days.

Table 4. In Vivo Activity of AbeAdo and Its Acetylated Derivative, **10**, against *T. b. rhodesiense* KETRI 243

compound	daily dose (mg/kg)	treatment time (days)	MSD[a] (days)	(no. cured)/total
control			8.8	0/5
AbeAdo	25 (pump)	7	31	2/5
	50 (pump)	7	31.3	2/5
	100 (pump)	7	32	4/5
control			13.4	0/5
10	10 (pump)	7	15.6	0/5
	25 (pump)	7	18.0	0/5
	50 (pump)	7	21.2	0/5

[a] MSD is the mean survival in days.

against KETRI 243 than AbeAdo (Table 1). The finding that **10** is less active than AbeAdo against KETRI 243 in vivo at the same doses (25 and 50 mg kg^{-1} day^{-1} in 7 day pumps) was unexpected. The dose−response relationship observed at the low concentrations tested suggested that at higher doses **10** might produce cures against KETRI 243. No further in vivo experiments were done to substantiate this prediction.

Since KETRI 243 isolate consists of a heterogeneous population with respect to drug resistance, derivative **10** was also examined for activity against four cloned subpopulations of KETRI 243. This agent, administered at 100 mg kg^{-1} day^{-1} (7 day pump), was 40−100% curative against clones selected for resistance to diamidines but only 20% curative against an arsenical-resistant clone (K243 As-10-3). Compound **10** was also

Table 5. In Vitro Antineoplastic Activity of AbeAdo Derivatives in Human and Murine Tumor Cell Lines

compound	cell line^a/IC$_{50}$ ^{b} (μM)					
	A121	A549	HT-29	MCF7	MCF7-Adr	L1210
AbeAdo	>100	$(3.0-100)^c$	>100	>100	200	
4	>100	>100	>100	>100	>100	>600
9	d	d	d	d	d	500
10	$(1-30) \pm 3.4^c$	$(3-10) \pm 2.8^c$	$(3-30) \pm 3.2^c$	51 ± 8.7	>100	20
14	41 ± 1.9	>100	112 ± 3.9	63 ± 6.1	78 ± 6.1	>600
16	>100	>100	>100	>100	>100	d
24	>100	>100	>100	>100	>100	>600
25	d	d	d	d	d	>600
36	>100	>100	>100	>100	>100	d

^{a} Cell lines are A121 (human ovarian carcinoma), A549 (human non-small-cell lung carcinoma), HT-29 (human colon adenocarcinoma), MCF7 (human breast adenocarcinoma), MCF7-Adr (adriamycin-resistant human breast adenocarcinoma), and L1210 (murine leukemia). ^{b} Concentration of drug necessary to inhibit cell growth by 50% after 72 h; see Experimental Section. ^{c} A plateau in cell growth was observed over a range of drug concentrations. This profile is typical of inhibitors of polyamine biosynthesis, since the cells are slowly depleted of polyamine pools. ^{d} Not determined.

curative against two other *T. b. rhodesiense* strains: KETRI 2002 and KETRI 2538. KETRI 2002 infected mice were cured by a daily dose of 100 mg/kg administered in a 7 day pump, while KETRI 2538 infected mice responded to ip dosing and 3 day pump regimens. In an earlier study, the parent compound AbeAdo had equivalent activity against both strains.

In Vitro Antineoplastic Activity. The in vitro antineoplastic activity of AbeAdo and its related analogues was determined against a variety of human tumor cell lines (A121 ovarian carcinoma, A549 non-small-cell lung carcinoma, HT-29 colon adenocarcinoma, MCF7-S breast adenocarcinoma, and MCF7-Adr, adriamycin-resistant breast carcinoma) and is shown in Table 5. In general, both AbeAdo and its analogues were devoid of significant antineoplastic activity against all the tumor cell lines assayed. However, as seen previously, acetylation of AbeAdo caused a significant increase (10-fold) in antineoplastic activity as evidenced by analogue **10** versus AbeAdo. As previously stated, the greater efficacy of AbeAdo as an inhibitor of trypanosomal growth, as opposed to mammalian cells, may be due to the active uptake of AbeAdo into trypanosomes via a purine transport system not found in mammalian cells.[11] Consequently, the enhanced antineoplastic activity of analogue **10** may be due to an increase in the passive uptake and activation of this analogue in mammalian cells.

In Vitro Enzyme Inhibitory Activity. To determine whether the acetylated analogues **10** and **16** could serve as substrates of the target enzyme, AdoMetDC, or were likely acting as prodrugs of AbeAdo, they were tested for their ability to inhibit AdoMetDC in vitro. The results are shown in Table 2. Although there is no direct correlation between the inhibition of AdoMetDC in vitro and antitrypanosomal activity, for the most part, inhibitors of this enzyme are also inhibitors of trypanosomal growth. Interestingly, the acetylated derivative of AbeAdo, **10**, is a substrate/inhibitor of AdoMetDC and may not be acting solely as a prodrug.

In Vitro Antiviral Activity. Polyamine pools, especially at the levels of putrescine, are elevated in the lymphocytes of patients with overt AIDS.[15] However, in another study, White et al.[16] did not observe elevated polyamine pools in HIV-1 infected CEM cells. Furthermore, AdoMetDC inhibitors such as MHZPA (5′-deoxy-5′-[(3-hydrazinopropyl)methylamino]-5′-adenosine) did not inhibit HIV-1 infectivity. Undaunted by these

Table 6. In Vitro Anti-HIV Activity of AbeAdo and Its Acetylated Derivative, **10**

compound	EC$_{50}$ (μM)^a	TC$_{50}$ (μM)^b	differential selectivity (TC$_{50}$/EC$_{50}$)
AbeAdo	0.93	<6.25	~6.7
10	0.05	12.0	240

^{a} EC$_{50}$ is the concentration required to inhibit plaque formation by 50%. ^{b} TC$_{50}$ is the concentration that causes morphological changes in noninfected CEM-SS cells.

results, the antiviral activity of AbeAdo and compound **10** was determined by use of a syncytial plaque assay in which HIV-1 infected CEM-SS cells, a syncytial clone, were treated with various concentrations of the sensitive compounds. The results, shown in Table 6, are expressed as EC$_{50}$ and TC$_{50}$ values. The EC$_{50}$ value is a measure of the concentration required to inhibit plaque formation by 50% (EC = effective concentration). The TC$_{50}$ value is a measure of the concentration that causes morphological changes in noninfected CEM-SS cells (TC = toxic concentration). The TC$_{50}$ value is predictive of cell cytotoxicity and inhibition of cell growth. Differential selectivity, defined as TC$_{50}$/EC$_{50}$, is used to identify potential anti-HIV compounds. An analogue with a differential selectivity greater than 100 is considered to be an effective inhibitor of HIV infectivity, as opposed to a nonspecific inhibitor of CEM-SS cell growth. The differential selectivity of AbeAdo (~6.7) suggests that its inhibition of HIV infectivity is due to nonspecific cytotoxicity in CEM-SS cells. In contrast, the differential selectivity of compound **10** is 240. Thus, compound **10** effectively inhibits HIV infectivity in CEM-SS cells at a concentration 240-fold less than that required to induce cytotoxicity in uninfected CEM-SS cells. The difference in activity between these two analogues will have to be further investigated.

Conclusions

The majority of structural modifications made to AbeAdo had a deleterious affect on both the antitrypanosomal and antineoplastic activity of this compound. However, di-O-acetylation of the parent compound produced **10**, a prodrug compound with markedly pronounced inhibition of trypanosomal and neoplastic cell growth and viability.

New structural inhibitors of AdoMet decarboxylase continue to be designed and synthesized as potential antitrypanosomal and antineoplastic agents.[26,27] Our

finding that compound **10** (but not its parent compound AbeAdo) inhibits HIV-1 growth and infectivity identifies a new target for possible therapeutic intervention by these nucleoside inhibitors of AdoMet decarboxylase.

Experimental Section

Human Tumor Cell Lines. The A121 ovarian carcinoma cell line was obtained from Dr. Kent Crickard, SUNY Buffalo.[17] Other human tumor cell lines including A549 non-small-cell lung carcinoma, HT-29 colon adenocarcinoma, MCF7-S breast adenocarcinoma. and MCF7-R adriamycin-resistant breast adenocarcinoma were purchased from the American Type Culture Collection. All cell lines are propagated as monolayers in RPMI-1640 containing 5% FCS, 5% NuSerum IV, 20 mM HEPES, and 2 mM L-glutamine at 37 °C in a 5% CO_2 humidified atmosphere. The doubling times for the cell lines range between 20 and 28 h.

Growth Inhibition Assay in 96-Well Microtiter Plates. Assessment of cell growth inhibition was determined according to the methods of Skehan et al.[18] Briefly, cells were plated between 400 and 1200 cells/well in 96-well plates and incubated at 37 °C 15−18 h prior to drug addition to allow attachment of cells. Compounds tested were solubilized in 100% DMSO and further diluted in RPMI-1640 containing 10 mM HEPES to a maximal final DMSO concentration of ≤0.25%, which is not growth inhibitory. Each cell line was treated with 10 concentrations of compound (5 log range). After a 72 h incubation, 100 µL of ice-cold 50% TCA was added to each well and incubated for 1 h at 4 °C. Plates were then washed 5 times with tap water to remove TCA, low-molecular-weight metabolites, and serum proteins. A 50 µL aliquot of 0.4% sulforhodamine B (SRB), an anionic protein stain, was added to each well. At cell densities ranging from very sparse to supraconfluent, SRB staining changes linearly with increases or decreases in number of cells and protein concentrations. These staining characteristics provide an accurate assessment of cell growth.[18] Following a 5 min incubation at room temperature, plates were rinsed 5 times with 0.1% acetic acid and were air-dried. Bound dye was solubilized with 10 mM Tris base (pH 10.5) for 5 min on a gyratory shaker. Optical density was measured at 570 nm.

Data Analysis. Data were fit with the Sigmoid-Emax concentration−effect model[19] with nonlinear regression, weighted by the reciprocal of the square of the predicted response. The fitting software was developed at RPCI with MicroSoft FORTRAN and uses the Marquardt[20] algorithm as adapted by Nash[21] for the nonlinear regression. The concentration of drug that inhibited growth by 50% (IC_{50}) was determined.

Trypanosome Strains. The *T. b. brucei* LAB 110 EATRO isolate was obtained from W. Trager of Rockefeller University.[22] Clinical isolates of *T. brucei rhodeiense* (KETRI 243 and KETRI 269) were obtained from A. R. Njogu of the Kenya Trypanosomiasis Research Institute (KETRI). The KETRI 243 isolate is resistant to diamidines and melarsoprol; KETRI 243 As 10-3 is a clone of KETRI 243, which is highly resistant to melarsoprol.[23] These strains were adapted to grow under axenic conditions in the bloodstream form in HMI-18 medium with hypoxanthine at 1 µM and 20% horse serum instead of synthetic serum.[24]

Determination of in Vitro Antitrypanosomal Activity. Drug studies were done in duplicate 24-well plates (1 mL per well), with final inhibitor concentrations of 0.1, 1, 10, 25, and 100 µM. After 48 h, the number of parasites was determined in a Z1 Coulter counter and the approximate range of activity was determined. The 50% inhibitory concentrations (IC_{50} values) were then determined from additional studies with closely spaced inhibitor concentrations. Inhibitors with <50% inhibition at 100 µM were not studied further. Most analogues were dissolved in water, and several were dissolved in 100% dimethyl sulfoxide. Dilutions were made with HMI-18 medium so that dimethyl sulfoxide concentrations never exceeded 0.3%, a noninhibitory concentration.

In Vivo Activity vs Trypanosome Mouse Model Infections. Mice (20 g of female Swiss-Webster) were infected with

2.5×10^5 trypanosomes from a rat infection. Compounds were dissolved in water. Treatment was begun 24 h after infection and consisted of twice per day (b.i.d.) intraperitoneal injections (9 a.m. and 4 p.m.) for 3 days. Alternatively, 7 day Alza miniosmotic pumps were loaded and implanted aseptically according to the manufacturer's instructions. Mice were monitored daily for viability, and tail vein blood smears were taken weekly and checked for trypanosomes. Animals surviving >30 days beyond the deaths of untreated control mice with no evidence of blood parasitemia were considered cured.

Determination of Antiviral Activity. This assay was conducted as previously reported.[25] Individual wells of a 96-well plate were coated with 50 µL of poly-L-lysine (50 µg/mL, MW = 90 000), incubated for 30 min at room temperature and washed 3 times with 0.01 M phosphate-buffered 0.85% (w/v) sodium chloride (PBS). Aliquots of CEM-SS cells in log-phase growth were washed 3 times with PBS, suspended at a density of 50 000 CEM-SS cells per 50 µL of RPMI-1640 medium without serum, transferred to the precoated wells, and incubated for 30 min at 37 °C in a humidified atmosphere containing 5% CO_2. The cell monolayer was then inoculated with 50 µL of HIV-1 (60−90 plaque forming units) diluted in growth medium. Compounds were formulated in growth medium for testing. After 120 min, the cell monolayer was overlaid with 100 µL of growth medium with or without test compounds and was incubated as before at 37 °C. Serial 2-fold dilutions of test compounds (0.00015−20 µM) were tested. After 3 days, each well received a second 100 µL overlay of growth medium with or without compound, and incubation was continued for an additional 2 days. On day 5, the syncytial plaques were counted and the concentration required to inhibit 50% of plaque formation (EC_{50}) was determined.

Synthetic Methods. ^{1}H NMR spectra were recorded on either a Bruker 400 MHz or Varian EM 390 spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Column chromatography was performed using silica gel 60 (230−400 mesh). TLC was done using EM Industries aluminum sheets (precoated with silica gel 60 F_{254}), and preparative TLC was done using Analtech uniplates (silica gel GF, 20 cm × 20 cm, and 1000 µm thick). The purity of all final compounds was determined by HPLC (McPherson FL75, C18 column, mobile phase CH_3CN/10 mM ammonium phosphate, pH 4.4) and verifed by elemental analysis (Robertson Microlit Laboratories, Inc. of Madison, NJ).

***cis*-*tert*-Butoxycarbonyl-4-chloro-2-butenyl-1-amine (1).** This intermediate was prepared according to literature procedures with analogous yields.[5]

5′-Deoxy-5′-chloroadenosine (2). This intermediate was prepared according to the procedure of Robbins et al.[12]

5′-Deoxy-5′-chloro-2′-deoxyadenosine (3). This intermediate was prepared according to the procedure of Robbins et al.[12]

5′-Deoxy-5′-methylaminoadenosine (4). 5′-Deoxy-5′-chloroadenosine (**2**) (5.224 g, 18.3 mmol) was chilled to −70 °C in a metal bomb, and 45 mL of condensed anhydrous methylamine was added. The bomb was sealed, heated to 55 °C for 72 h, and then cooled to −70 °C. The bomb was unsealed and allowed to come slowly to room temperature. After the excess methylamine had boiled off, the resulting thick oil was taken up in H_2O (50 mL) and allowed to stand for 18 h at room temperature. The mixture was filtered to remove unreacted **2**, and the filtrate was concentrated in vacuo. The thick oil was then extracted with EtOAc/H_2O (11:1) until TLC (CH_2Cl_2/MeOH, 5:1) indicated only product was present. The thick oil was dried under vacuum to give **4** (2.489 g, 49%) as a hygroscopic solid. ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3-NH), 3.30 (m, 2H, 5′-CH_2), 4.25 (m, 2H, 2′- and 3′-CH), 4.75 (m, 1H, 4′-CH), 5.65 (br m, 2H, OH), 5.90 (m, 1H, 1′-CH), 7.25 (s, 2H, NH_2), 8.15 (s, 1H, Ad-H8), and 8.40 (s, 1H, Ad-H2). Anal. ($C_{11}H_{16}N_6O_3$·1.25H_2O) C, H, N.

5′-Deoxy-5′-methylamino-2′-deoxyadenosine (5). This intermediate was prepared from compound **3** (0.186 g, 0.7 mmol) and 25 mL of anhydrous methylamine (96 h) in a manner analogous to that of **4**. The crude product was purified

on preparative silica gel plates developed in CH_2Cl_2/MeOH (4:1). The appropriate bands were scraped and stirred with 100 mL of CH_2Cl_2/MeOH (7:3), the silica gel was filtered, and the filtrate was concentrated in vacuo to give 0.119 g (65%) of product.

cis-5′-Deoxy-5′-(4-*tert*-butoxycarbonylamino-3-butenyl)-methylaminoadenosine (6). A solution of **4** (0.228 g, 0.81 mmol), **1** (0.182 g, 0.88 mmol), K_2CO_3 (0.113 g, 0.82 mmol), and NaI (0.123 g, 0.82 mmol) in DMF (10 mL) was heated to 85 °C for 72 h. The reaction mixture was cooled to room temperature, diluted with H_2O (50 mL), and extracted with CH_2Cl_2 until TLC (CH_2Cl_2/MeOH, 4:1) indicated the absence of product in the aqueous layer. The CH_2Cl_2 extracts were pooled, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (continuous gradient of CH_2Cl_2/MeOH, 4:1, and 2% triethylamine) and the appropriate fractions were pooled, treated with charcoal, filtered through Celite, and concentrated in vacuo to give 0.248 g (69%) of product as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.45 [s, 9H, $C(CH_3)_3$], 2.50 (s, 3H, CH_3NH), 3.15 (m, 2H, 5′-CH_2), 3.75 (m, 2H, CH_2NHCH), 4.25 (m, 4H, 2′- and 3′-CH, t-BOC−NH−CH_2), 4.75 (m, 1H, 4′-CH), 5.65 (br m, 2H, OH), 5.50 (m, 2H, CH=CH), 5.95 (m, 1H, 1′-CH), 6.6−6.9 (m, 3H, NH_2, NH), 8.05 (s, 1H, Ad-H8), and 8.20 (s, 1H, Ad-H2).

cis-5′-Deoxy-5′-(4-*tert*-butoxycarbonylamino-3-butenyl)-methylamino-2′-deoxyadenosine (7). This intermediate was prepared in an analogous manner to that of **6**. Compound **5** (0.113 g, 0.43 mmol), **1** (0.111 g, 0.54 mmol), K_2CO_3 (0.071 g, 0.51 mmol), and NaI (0.077 g, 0.51 mmol) in DMF (15 mL) were heated to 85 °C for 72 h. The crude product was applied to preparative silica gel plates and was developed with CH_2Cl_2/MeOH (3:1) and the appropriate bands were scraped, stirred with CH_2Cl_2/MeOH (7:3), filtered, and concentrated in vacuo to give 0.099 g (53%) of product. ^{1}H NMR (CD_3OD): δ 1.45 [s, 9H, $C(CH_3)_3$], 2.30 (overlapping m, 5H, 2′-CH_2, CH_3-NH), 2.75 (m, 2H, 5′-CH_2), 3.20 (m, 2H, CH_2NHCH), 3.60 (m, 2H, t-BOC−NH−CH_2), 4.1−4.4 (m, 2H, 3′- and 4′-CH), 5.50 (m, 2H, CH=CH), 6.35 (m, 1H, 1′-CH), 8.20 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

cis-5′-Deoxy-5′-(4-*tert*-butoxycarbonylamino-3-butenyl)-methylamino-2′,3′-di-*O*-acetyladenosine (8). A solution of **6** (0.248 g, 0.55 mmol), acetic anhydride (125 μL, 1.32 mmol), triethylamine (208 μL, 1.45 mmol), and 4-(dimethylamino)-pyridine (5 mg, 0.04 mmol) in CH_3CN (10 mL) was stirred at room temperature for 3 h. MeOH (5 mL) was added to the reaction mixture, and the solution was stirred an additional 30 min. The reaction mixture was concentrated in vacuo, and the resulting oil was taken up in CH_2Cl_2 (50 mL), washed once× with saturated $NaHCO_3$ (50 mL) and once with H_2O (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was applied to two preparative silica gel plates and was developed in CH_2Cl_2/MeOH (4:1). The appropriate bands were scraped, stirred with CH_2Cl_2/MeOH (7:3), and filtered, and the filtrate was concentrated in vacuo to yield 0.211 g (72%) of product as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.45 [s, 9H, $C(CH_3)_3$], 2.10 [d, 6H, $(COCH_3)_2$], 2.25 (s, 3H, CH_3NH), 2.80 (m, 2H, 5′-CH_2), 3.10 (m, 2H, CH_2NHCH), 3.80 (m, 2H, t-BOC−NH−CH_2), 4.30 (m, 2H, 2′- and 3′-CH), 4.95 (m, 1H, 4′-CH), 5.50 (m, 2H, CH=CH), 5.90−6.10 (m, 4H, 1′-CH, NH_2, NH), 8.00 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

cis-5′-Deoxy-5′-(4-amino-2-butenyl)methylamino-2′-deoxyadenosine (9). This intermediate was prepared from **7** (0.118 g, 0.28 mmol) and trifluoroacetic acid/CH_2Cl_2 (1:1, 8 mL) in a manner analogous to that of **10**. The reaction mixture was concentrated in vacuo and azeotroped 3× with CH_2Cl_2. The resulting orange oil was taken up in MeOH and treated with charcoal, and the solution was filtered (0.2 μm) and concentrated in vacuo. The pale-yellow oil was precipitated 4× from MeOH and ether, and the product was taken up in MeOH, decolorized with charcoal, filtered (0.2 μm) into a tared vial, and dried to constant weight to give 78 mg (60%) of product as a very hygroscopic solid. ^{1}H NMR (DMSO-d_6): δ 2.30 (m, 2H, 2′-CH_2), 2.55 (s, 3H, CH_3NH), 3.35 (m, 2H, 5′-CH_2), 3.50−3.65 (m, 4H, CH_2NHCH=CHCH_2), 4.15−4.45 (m, 2H, 3′- and 4′-CH), 5.85 (m, 2H, CH=CH), 6.40 (m, 1H, 1′-CH), 7.3 (s, 2H, NH_2), 8.15 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2). MS: (M + H) 334.5, (M + H + 1.2TFA) 467.9.

cis-5′-Deoxy-5′-(4-amino-2-butenyl)methylamino-2′,3′-di-*O*-acetyladenosine (10). A solution of **8** (0.107 g, 0.2 mmol) in trifluoroacetic acid/CH_2Cl_2 (1:1) was allowed to stand at room temperature for 90 min. The reaction mixture was concentrated in vacuo and coevaporated 3× with CH_2Cl_2. The resulting oil was taken up in 5 mL of CH_2Cl_2, 1 mL of MeOH, and 50 mL of ether and placed at −20 °C for 18 h. The solution was decanted, and the precipitate was tritrated 2× with ether. The white solid was dried under vacuum to give 0.010 g (66%) of product. ^{1}H NMR (CD_3OD): δ 2.00 [d, 6H, $(COCH_3)_2$], 2.80 (s, 3H, CH_3NH), 3.30−3.95 (m, 6H, 5′-CH_2−N-CH_2−CH=CH-CH_2−NH_3), 4.30 (m, 2H, 2′- and 3′-CH), 4.95 (m, 1H, 4′-CH), 5.95 (m, 2H, CH=CH), 6.25 (m, 1H, 1′-CH), 8.25 (m, 2H, Ad-H8 and Ad-H2). Anal. ($C_{19}H_{28}N_7O_5$·$^{21}/_2$CF$_3$COOH·CH_3OH) C, H, N, F.

5′-Deoxy-5′-chloro-2′,3′-seco-adenosine (11). This intermediate was prepared as previously reported from **2** (1.30 g, 4.6 mmol) to yield 1.14 g of product as a waxy solid (86%).[12] ^{1}H NMR (DMSO-d_6): δ 3.40−3.90 (m, 7H, 2′,3′-CH_2, 4′-CH, 5′-CH_2), 5.10 (br m, 2H, HO), 5.90 (t, 1H, 1′-CH), 7.20 (m, 2H, NH_2), and 8.20−8.40 (m, 2H, Ad-H2 and H8).

5′-Deoxy-5′-methylamino-2′,3′-seco-adenosine (12). This intermediate was prepared from **11** (0.348 g, 1.2 mmol) and anhydrous methylamine (8 mL) in a manner analogous to that of **4**. The crude product was purified on preparative silica gel plates developed in CH_2Cl_2/MeOH (4:1). The appropriate bands were scraped and stirred with 100 mL of CH_2Cl_2/MeOH (7:3). The silica gel was filtered, and the filtrate was concentrated in vacuo to give 0.105 g (31%) of product. ^{1}H NMR (CD_3OD-d_6): δ 2.30 (s, 3H, CH_3NH), 2.80 (m, 2H, 5′-CH_2), 3.75−4.15 (m, 5H, 2′,3′-CH_2, 4′-CH), 6.10 (t, 1H, 1′-CH), and 8.20−8.40 (m, 2H, Ad-H2 and H8).

cis-5′-Deoxy-5′-(4-*tert*-butoxycarbonylamino-3-butenyl)-methylamino-2′,3′-seco-adenosine (13). This intermediate was prepared from **12** (0.105 g, 0.37 mmol), **1** (0.091 g, 0.44 mmol), K_2CO_3 (0.057 g, 0.41 mmol), and NaI (0.061 g, 0.41 mmol) in DMF (15 mL), and the mixture was heated to 85 °C for 72 h in a manner analogous to that of **6**. The crude product was applied to preparative silica gel plates and was developed with CH_2Cl_2/MeOH (2.5:1) and the appropriate bands were scraped, stirred with CH_2Cl_2/MeOH (7:3), filtered, and concentrated in vacuo to give 0.048 g (29%) of product. ^{1}H NMR (DMSO-d_6): δ 1.30 [s, 9H, $C(CH_3)_3$], 2.50−2.70 (s, 5H, CH_3-NH, 5′-CH_2), 3.30−3.90 (m, 9H, 2′, 3′-CH_2, 4′-CH, CH_2CH=CHCH_2), 5.10−5.40 (m, 4H, CH=CH, OH), 5.90 (t, 1H, 1′-CH), 7.20 (s, 2H, NH_2), and 8.10−8.30 (m, 2H, Ad-H2 and H8).

cis-5′-Deoxy-5′-(4-amino-2-butenyl)methylamino-2′,3′-seco-adenosine (14). This intermediate was prepared from **13** (0.048 g, 0.10 mmol) in trifluoroacetic acid/CH_2Cl_2 (1:1, 2 mL) in a manner analogous to that of **9** to afford 0.056 g (88%) of product as a pale-yellow hygroscopic solid. ^{1}H NMR (DMSO-d_6): δ 2.50−2.60 (m, 5H, CH_3NH, 5′-CH_2), 3.60−4.10 (m, 9H, 2′,3′-CH_2, 4′-CH, CH_2CH=CHCH_2), 5.35 (br m, 2H, OH), 5.85 (m, 2H, CH=CH), 6.00 (t, 1H, 1′-CH), 7.50 (s, 2H, NH_2), 8.15 (m, 2H, NH_2), and 8.20−8.50 (m, 2H, Ad-H2 and H8). Anal. ($C_{15}H_{24}N_7O_3$·1.2CF$_3$COOH·0.5CH_3OH·H_2O) C, H, N, F.

cis-5′-Deoxy-5′-(4-*tert*-butoxycarbonylamino-3-butenyl)-methylamino-2′,3′-di-*O*-acetyl-seco-adenosine (15). This intermediate was prepared from **13** (0.07 g, 0.16 mmol), acetic anhydride (360 μL, 0.38 mmol), triethylamine (545 μL, 0.38 mmol), and (dimethylamino)pyridine (0.002 g, 0.08 mmol) in CH_3CN (20 mL) in a manner analogous to that of **8**. The crude product was applied to preparative silica gel plates and was developed in CH_2Cl_2/MeOH (6:1). The appropriate bands were scraped, stirred with CH_2Cl_2/MeOH (7:3), and concentrated in vacuo to give 0.068 g (79%) of product. ^{1}H NMR ($CDCl_3$): δ 1.80−2.30 (m, 9H, CH_3NH, 2′,3-CH_3CO), 2.80 (m, 2H, 5′-CH_2), 3.40−4.50 (m, 9H, 2′,3′-CH_2, 4′-CH, CH_2CH=CHCH_2), 5.50 (m, 2H, NH_2), 6.00 (m, 2H, CH=CH), 6.20 (t, 1H, 1′-CH), and 8.10−8.30 (m, 2H, Ad-H2 and H8).

cis-**5′-Deoxy-5′-(4-amino-2-butenyl)methylamino-2′,3′-di-*O*-acetyl-*seco*-adenosine (16).** This congener was prepared from **15** (0.068 g, 0.13 mmol) in trifluoroacetic acid/CH$_2$Cl$_2$ (1:1, 6 mL) in a manner analogous to that of **10** to afford 0.070 g (76%) of product as a hygroscopic solid. ^{1}H NMR (DMSO-d_6): δ 1.90−2.10 (m, 6H, 2′,3-CH$_3$CO), 2.50 (s, 3H, CH$_3$NH), 3.15 (m, 2H, 5′-CH$_2$), 3.50 (br m, 8H, *CH$_2$*CH= CH*CH$_2$*, 2′,3′-CH$_2$), 4.70 (m, 1H, 4′-CH), 5.60−5.80 (m, 2H, CH=CH), 6.20 (t, 1H, 1′-CH), 7.50 (br s, 2H, NH$_2$), 8.10 (br s, 2H, NH$_2$), and 8.20−8.40 (m, 2H, Ad-H2 and H8). Anal. (C$_{19}$H$_{30}$N$_7$O$_5$·2.5CF$_3$COOH·0.75H$_2$O) C, H, N, F.

5′-Deoxy-5′-chloro-2′,3′-isopropylideneadenosine (18). This intermediate was prepared according to the procedure of Robbins et al.[11]

5′-Deoxy-5′-methylamino-2′,3′-isopropylideneadenosine (19). This intermediate was prepared from **17** (0.569 g, 1.75 mmol) and anhydrous methylamine (15 mL) in a manner analogous to that of **3**. The crude product was purified on preparative silica gel plates developed in CH$_2$Cl$_2$/MeOH (7:1). The appropriate bands were scraped and stirred with 100 mL of CH$_2$Cl$_2$/MeOH (7:3), the silica gel was filtered, and the filtrate was concentrated in vacuo to give 0.392 g (63%) of product. ^{1}H NMR (CDCl$_3$/CD$_3$OD): δ 1.4−1.6 [2s, 6H, C(CH$_3$)$_3$], 2.50 (s, 3H, CH$_3$NH), 3.30 (m, 2H, 5′-CH$_2$,), 4.50 (m, 1H, 4′-CH), 5.10−5.50 (m, 2H, 2′- and 3′-CH), 6.10 (m, 2H, 1′-CH), 8.00 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

5′-Deoxy-5′-(*p*-toluenitrile)methylamino-2′,3′-isopropylideneadenosine (20). This intermediate was prepared in a manner analogous to that of **6**. Compound **19** (0.392 g, 1.1 mmol), α-bromo-*p*-toluenitrile (0.237 g, 1.2 mmol), K$_2$CO$_3$ (0.166 g, 1.2 mmol), and NaI (0.181 g, 1.2 mmol) were placed in CH$_3$CN (25 mL) and refluxed for 18 h. The reaction mixture was cooled to room temperature, diluted with 100 mL of EtOAc, and washed with brine, and the organic layer was dried over MgSO$_4$, filtered, and concentrated in vacuo. The crude product was applied to preparative silica gel plates and was developed with CH$_2$Cl$_2$/MeOH (7:1). The appropriate bands were scraped, stirred with CH$_2$Cl$_2$/MeOH (7:3), filtered, and concentrated in vacuo to give 0.281 g (54%) of a clear waxy solid. ^{1}H NMR (CDCl$_3$): δ 1.4−1.6 [2s, 6H, C(CH$_3$)$_3$], 2.30 (s, 3H, CH$_3$NH), 3.50 (m, 2H, 5′-CH$_2$,), 4.50 (m, 1H, 4′-CH), 5.00− 5.50 (m, 2H, 2′- and 3′-CH), 5.85 (m, 2H, CH$_2$-toluenitrile), 6.10 (m, 2H, 1′-CH), 7.40 (m, 4H, aromatic Hs of toluenitrile), 8.00 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

5′-Deoxy-5′-(*o*-toluenitrile)methylamino-2′,3′-isopropylideneadenosine (21). This intermediate was prepared in a manner analogous to that of **20**. Compound **19** (0.472 g, 1.3 mmol), α-bromo-*o*-toluenitrile (0.317 g, 1.6 mmol), K$_2$CO$_3$ (0.224 g, 1.6 mmol), and NaI (0.241 g, 1.6 mmol) were placed in CH$_3$CN (25 mL) and refluxed for 18 h. The reaction mixture was cooled to room temperature, diluted with 100 mL of EtOAc, and washed with brine, and the organic layer was dried over MgSO$_4$, filtered, and concentrated in vacuo. The crude product was applied to a silica gel column packed in EtOAc and eluted isocratically with EtOAc/MeOH (25:1). The appropriate fractions were pooled, concentrated in vacuo, and dried under vacuum to give 0.481 g (77%) of product. ^{1}H NMR (CDCl$_3$) δ 1.4−1.6 [2s, 6H, C(CH$_3$)$_3$], 2.30 (s, 3H, CH$_3$NH), 3.65 (m, 2H, 5′-CH$_2$), 4.45 (m, 1H, 4′-CH), 5.00−5.50 (m, 2H, 2′- and 3′-CH), 6.10 (m, 2H, CH$_2$-toluenitrile), 6.20 (m, 2H, 1′-CH), 7.40 (m, 4H, aromatic Hs of toluenitrile), 7.90 (s, 1H, Ad-H8), and 8.25 (s, 1H, Ad-H2).

5′-Deoxy-5′-(*p*-aminomethylphenyl)methylamino-2′,3′-isopropylideneadenosine (22). A solution of **20** (0.281 g, 0.59 mmol) in anhydrous THF (10 mL) was added dropwise to a solution of LiAlH$_4$ (0.035 g, 0.88 mmol) in anhydrous THF (20 mL) under an inert atmosphere. The solution was stirred at room temperature for 2 h, at which time TLC indicated the presence of starting material (∼40%). An additional 0.88 mmol of hydride was added to the reaction mixture. After an additional 2 h, TLC still indicated the presence of starting material (∼20%). Another 0.88 mmol of hydride was added to the reaction mixture. After an additional hour, TLC indicated the absence of starting material. Unreacted hydride was

decomposed by the slow addition of H$_2$O. The reaction mixture was then diluted with H$_2$O (100 mL) and allowed to stand at room temperature overnight. The solution was filtered to remove inorganic salts, and the filtrate was concentrated in vacuo. The resulting thick oil was taken up in a minimum amount of MeOH to further remove inorganic salts and was filtered, and the filtrate was concentrated in vacuo. The crude product was applied to preparative silica gel plates and was developed with CH$_2$Cl$_2$/MeOH/Et$_3$N (20:5:0.5). The appropriate bands were scraped, stirred with CH$_2$Cl$_2$/MeOH (7:3) and 2% Et$_3$N (v/v), filtered, and concentrated in vacuo to give 0.146 g (52%) of a white, foamy solid. ^{1}H NMR (CD$_3$OD): δ 1.4−1.6 [2s, 6H, C(CH$_3$)$_3$], 2.25 (s, 3H, CH$_3$NH), 2.50 (m, 2H, H$_2$NCH$_2$), 3.35 (m, 2H, 5′-CH$_2$), 3.85 [m, 2H, CH$_2$−N(CH$_3$)CH], 4.30 (m, 1H, 4′-CH), 4.90−5.30 (m, 2H, 2′- and 3′-CH), 6.10 (m, 2H, 1′-CH), 7.10 (s, 4H, aromatic Hs of phenyl), 7.90 (s, 1H, Ad-H8), and 8.10 (s, 1H, Ad-H2).

5′-Deoxy-5′-(*o*-aminomethylphenyl)methylamino-2′,3′-isopropylideneadenosine (23). This intermediate was prepared from **21** (0.481 g, 1.0 mmol) and LiAlH$_4$ (0.128 g, 3.0 mmol) in a manner analogous to that of **22**. The crude product was applied to a silica gel column packed in CH$_2$Cl$_2$/MeOH (16:1 + 5% Et$_3$N v/v) and eluted with a continuous gradient to 2:1 + 5% Et$_3$N v/v. The appropriate fractions were pooled, concentrated in vacuo, and dried under vacuum to give 0.133 g (28%) of product as a foamy white solid. ^{1}H NMR (CD$_3$OD): δ 1.4−1.6 [2s, 6H, C(CH$_3$)$_3$], 2.25 (s, 3H, CH$_3$NH), 2.55 (m, 2H, H$_2$NCH$_2$), 3.50 (m, 2H, 5′-CH$_2$), 3.75 [m, 2H, CH$_2$−N(CH$_3$)-CH], 4.30 (m, 1H, 4′-CH), 4.90−5.30 (m, 2H, 2′- and 3′-CH), 6.10 (m, 2H, 1′-CH), 7.10 (s, 4H, aromatic Hs of phenyl), 7.90 (s, 1H, Ad-H8), and 8.10 (s, 1H, Ad-H2).

5′-Deoxy-5′-(*p*-aminomethylphenyl)methylaminoadenosine (24). Compound **22** (0.141 g, 0.3 mmol) was taken up in 1.0 N H$_2$SO$_4$ (5 mL) and was allowed to stand at room temperature for 18 h. The solution was diluted with 200 mL of EtOH and placed at −20° C for 18 h. The solution was decanted, and the precipitate was redissolved in a minimum amount of H$_2$O, diluted with 200 mL of EtOH, and placed at −20° C for 18 h. The solution was decanted, and the solid was dissolved in MeOH, transferred to a tared vial, and dried under vacuum to constant weight to give 0.133 g (65%) of product as a white hygroscopic powder. ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH$_3$NH), 2.50 (m, 2H, H$_2$NCH$_2$), 3.45 (m, 2H, 5′-CH$_2$), 3.85 [m, 2H, CH$_2$−N(CH$_3$)CH], 4.20−4.50 (m, 3H, 2′-, 3′-, 4′-CH), 5.40 (m, 1H, OH), 5.80 (m, 1H, OH), 6.10 (m, 1H, 1′-CH), 6.30 (bs, 2H, NH$_2$), 7.10−7.30 (m, 6H, aromatic Hs of phenyl, NH$_2$), 7.90 (s, 1H, Ad-H8), and 8.10 (s, 1H, Ad-H2). Anal. (C$_{19}$H$_{26}$N$_7$O$_3$· 2.3H$_2$SO$_4$·1.5CH$_3$OH·1H$_2$O) C, H, N, S.

5′-Deoxy-5′-(*o*-aminomethylphenyl)methylaminoadenosine (25). This intermediate was prepared from **23** (0.113 g, 0.24 mmol) in 1 N H$_2$SO$_4$ (5 mL) in a manner analogous to that of **23**. The product was isolated as previously detailed to yield 0.102 g (69%) of product as a white hygroscopic powder. ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H, CH$_3$NH), 2.50 (m, 2H, H$_2$-NCH$_2$), 3.50 (m, 2H, 5′-CH$_2$), 3.80 [m, 2H, CH$_2$−N(CH$_3$)CH], 4.25−4.45 (m, 3H, 2′-, 3′-, 4′-CH), 5.60 (m, 1H, OH), 5.80 (m, 1H, OH), 6.10 (m, 1H, 1′-CH), 6.45 (bs, 2H, NH$_2$), 7.10 (s, 4H, aromatic Hs of phenyl), 7.40 (bs, 2H, NH$_2$), 7.90 (s, 1H, Ad-H8), and 8.10 (s, 1H, Ad-H2). Anal. (C$_{19}$H$_{26}$N$_7$O$_3$·1.9H$_2$SO$_4$·CH$_3$-OH) C, H, N, S.

2′,3′-Isopropylidene-2-aminoadenosine (26). To a solution of 2-aminoadenosine (0.793 g, 2.8 mmol) in acetone (340 mL) and 2,2-dimethoxypropane (1 mL) was added 70% perchloric acid (1.3 mL). The solution was stirred at room temperature for 1 h and was neutralized by the addition of pyridine. The solution was concentrated in vacuo, and the resulting oil was taken up in 15 mL of MeOH and allowed to stand for 18 h at room temperature. The solution was filtered, and the filtrate was concentrated in vacuo. The obtained crude product was purified on preparative silica gel plates developed in CH$_2$Cl$_2$/MeOH (8:1). The appropriate bands were scraped and stirred with 100 mL of CH$_2$Cl$_2$/MeOH (7:3), the silica gel was filtered, and the filtrate was concentrated in vacuo to give 0.873 g (97%) of product as a foamy solid. ^{1}H NMR (DMSO-

d_6): δ 1.3−1.5 [2s, 6H, C(CH$_3$)$_3$], 2.50 (m, 1H, OH), 3.55 (m, 2H, 5′-CH$_2$), 4.20 (m, 1H, 4′-CH), 5.00−5.30 (m, 2H, 2′- and 3′-CH), 5.95 (m, 1H, 1′-CH), 7.50 (m, 2H, NH$_2$), 8.20 (s, 1H, 8-CH), and 8.45 (m, 2H, NH$_2$).

5′-Deoxy-5′-chloro-2′,3′-isopropylidene-2-aminoadenosine (27). This intermediate was prepared according to the procedure of Robbins et al.[11] ^{1}H NMR (CDCl$_3$): δ 1.3−1.5 [2s, 6H, C(CH$_3$)$_3$], 3.80 (m, 1H, 4′-CH), 4.40 (m, 2H, 5′-CH$_2$), 4.95 (m, 2H, NH$_2$), 5.10−5.40 (m, 2H, 2′- and 3′-CH), 5.95 (m, 1H, 1′-CH), 6.10 (2, 2H, NH$_2$), and 7.55 (s, 1H, 8-CH).

5′-Deoxy-5′-methylamino-2′,3′-isopropylidene-2-aminoadenosine (28). This intermediate was prepared from **27** (0.109 g, 0.36 mmol) and anhydrous methylamine (20 mL, reaction time 96 h) in a manner analogous to that of **4**. The crude product was purified on preparative silica gel plates developed in CH$_2$Cl$_2$/MeOH (5:1). Appropriate bands were scraped and stirred with 100 mL of CH$_2$Cl$_2$/MeOH (7:3), the silica gel was filtered, and the filtrate was concentrated in vacuo to give 0.134 g of product. ^{1}H NMR (CD$_3$OD): δ 1.3−1.5 [2s, 6H, C(CH$_3$)$_3$], 2.55 (s, 3H, CH$_3$NH), 3.10 (m, 2H, 5′-CH$_2$), 3.60 (m, 1H, 4′-CH), 5.00−5.30 (m, 2H, 2′- and 3′-CH), 6.00 (m, 1H, 1′-CH), and 7.80 (s, 1H, 8-CH).

cis-5′-Deoxy-5′-(4-tert-butoxycarbonylamino-3-butenyl)methylamino−2′,3′-isopropylidene-2-aminoadenosine (29). This intermediate, prepared from **28** (0.134 g, 0.40 mmol), **1** (0.093 g, 0.46 mmol), K$_2$CO$_3$ (0.069 g, 0.46 mmol), and NaI (0.074 g, 0.50 mmol) in CH$_3$CN (25 mL), was refluxed for 18 h in a manner analogous to that of **6**. The crude product was applied to preparative silica gel plates and was developed with CH$_2$Cl$_2$/MeOH (1:1) and the appropriate bands were scraped, stirred with CH$_2$Cl$_2$/MeOH (7:3), filtered, and concentrated in vacuo to give 0.058 g (29%) of product. ^{1}H NMR (CDCl$_3$): δ 1.45 [s, 12H, C(CH$_3$)$_3$, C−CH$_3$], 1.51 (s, 3H, C−CH$_3$), 2.40 (s, 3H, CH$_3$NH), 3.15 (m, 2H, 5′-CH$_2$), 3.75 (m, 2H, CH$_2$NHCH), 4.30 (m, 4H, 2′- and 3′-CH, t-BOC−NH−CH$_2$), 4.85 (m, 1H, 4′-CH), 5.50 (m, 2H, CH=CH), 5.95 (m, 1H, 1′-CH), and 7.65 (s, 1H, Ad-H8).

cis-5′-Deoxy-5′-(4-amino-2-butenyl)methylamino-2-aminoadenosine (30). This intermediate was prepared from **29** (0.0.58 g, 0.12 mmol) in 1 N H$_2$SO$_4$ (5 mL) in a manner analogous to that of **24**. The product was isolated as previously detailed to yield 0.053 g (69%) of product as a white hygroscopic powder. NMR (DMSO-d_6): δ 2.40 (s, 3H, CH$_3$NH), 3.15 (m, 2H, 5′-CH$_2$), 3.45−3.70 (m, 4H, CH$_2$NHCH=CHCH$_2$), 4.20−4.40 (m, 2H, 2′- and 3′-CH), 4.55 (m, 1H, 4′-CH), 5.70 (m, 2H, CH=CH), 6.0 (m, 1H, 1′-CH), 6.80 (bs, 2H, NH$_2$), 7.25 (bs, 2H, NH$_2$), and 7.65 (s, 1H, Ad-H8). Anal. (C$_{15}$H$_{25}$N$_8$O$_3$·2.3H$_2$-SO$_4$·1.5CH$_3$CH$_2$OH) C, H, N, S.

9-(Acetoxyethylmethoxy)adenine (31). This intermediate was synthesized according to the procedure of Robbins et al.[12] ^{1}H NMR (CDCl$_3$): δ 2.00 (s, 3H, CH3), 3.85 (m, 2H, AcOCH$_2$CH$_2$O), 4.30 (m, 2H, AcOCH$_2$CH$_2$O), 5.70 (s, 2H, OCH$_2$-Ad), 8.20 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

9-(Hydroxyethylmethoxy)adenine (32). Compound **27** was suspended in MeOH (20 mL). To this solution was added 1 M sodium methoxide (1.7 mL), and the solution was allowed to stir at room temperature for 2 h. Several grams of silica gel were added to the reaction mixture, and the solution was then concentrated in vacuo. The crude product, once absorbed onto silica gel, was placed on top of a silica gel column packed in CH$_2$Cl$_2$ and eluted with 150 mL portions of CH$_2$Cl$_2$/MeOH (from 100:0 to 95:5) and then isocratically at 94:6 until all desired product eluted from the column. The appropriate fractions were pooled, concentrated in vacuo, and dried under vacuum to give 0.991 g (99%) of product as a white powder. ^{1}H NMR (DMSO-d_6): δ 3.35−3.50 (m, 4H, AcOCH$_2$CH$_2$O), 4.65 (m, 1H, HO), 5.60 (s, 2H, OCH$_2$-Ad), 8.20 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

9-(Chloroethylmethoxy)adenine (33). CCl$_4$ (664 μL, 7.0 mmol) was added dropwise to a solution of **32** (0.972 g, 4.65 mmol) and triphenylphosphine (2.444 g, 9.30 mmol) in anhydrous pyridine (80 mL). The reaction mixture was stirred overnight at room temperature under an inert atmosphere of argon. The reaction mixture was concentrated in vacuo, taken

up into MeOH, absorbed onto silica gel, and then applied to the top of a silica gel column packed in CH$_2$Cl$_2$. The column was eluted with 150 mL portions of CH$_2$Cl$_2$/MeOH (from 100:0 to 95:5) and then isocratically at 94:6 until all desired product eluted from the column. The appropriate fractions were pooled, concentrated in vacuo, and dried under vacuum to give 0.948 g (85%) of product. ^{1}H NMR (DMSO-d_6): δ 3.25 (m, 2H, ClCH$_2$CH$_2$O), 3.65 (m, 2H, ClCH$_2$CH$_2$O), 5.60 (s, 2H, OCH$_2$-Ad), 8.20 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

9-(Methylaminoethylmethoxy)adenine (34). This intermediate was prepared from **33** (0.109 g, 0.36 mmol) in anhydrous methylamine (20 mL) in a manner analogous to that of **4** (60 °C for 72 h). The excess methylamine was evaporated, the crude product was then tritrated 3× with CH$_2$-Cl$_2$, and the resulting oil was dried under vacuum to give 1.116 g (92%) of a white hygroscopic foam. ^{1}H NMR (DMSO-d_6): δ 2.55 (s, 3H, CH$_3$NH), 3.15 (m, 2H, HNCH$_2$CH$_2$O), 3.85 (m, 2H, HNCH$_2$CH$_2$O), 5.65 (s, 2H, OCH$_2$-Ad), 8.20 (s, 1H, Ad-H8), and 8.30 (s, 1H, Ad-H2).

cis-(4-tert-Butoxycarbonylamino-3-butenyl)methylamino(ethylmethoxy)adenine (35). This intermediate was prepared from **34** (0.522 g, 2.5 mmol), **1** (0.758 g, 3.8 mmol), K$_2$CO$_3$ (0.494 g, 3.6 mmol), and NaI (0.531 g, 3.5 mmol) in DMF (20 mL) heated to 85 °C for 48 h in a manner analogous to that of **6**. The reaction mixture was concentrated in vacuo. The crude product was applied to a silica gel column packed in CH$_2$Cl$_2$ and eluted with 150 mL portions of CH$_2$Cl$_2$/MeOH (from 100:0 to 91:9) and then isocratically at 90:10 until all desired product eluted from the column. The appropriate fractions were pooled, concentrated in vacuo, and dried under vacuum to give 0.183 g (19%) of product as a waxy solid. ^{1}H NMR (CDCl$_3$): δ 1.45 [s, 12H, C(CH$_3$)$_3$], 2.45 (s, 3H, CH$_3$NH), 2.95 (m, 2H, NCH$_2$CH$_2$O), 3.45 (m, 2H, t-BOC−NHCH$_2$), 3.80 (m, 4H, CH=CHCH$_2$NCH$_2$-CH$_2$O), 5.65 (m, 4H, CH=CH, OCH$_2$-Ad), 8.15 (s, 1H, Ad-H8), 8.30 (s, 1H, Ad-H2).

cis-(4-Amino-2-butenyl)methylamino(ethylmethoxy)adenine (36). This intermediate was prepared from **35** (0.162 g, 0.40 mmol) in 1 N H$_2$SO$_4$ (2 mL) in a manner analogous to that of **24**. The reaction mixture was diluted with EtOH (100 mL), and a minimum amount of H$_2$O/MeOH was added to achieve a homogeneous mixture. The solution was further diluted with 50 mL of ether and placed at −20 °C for 18 h. The solution was decanted, and the precipitate was redissolved in H$_2$O (2 mL), EtOH (150 mL), and ether (100 mL) and was placed at −20 °C for 18 h. The solution was decanted, and the precipitate was dissolved in H$_2$O, filtered (0.2 μm), concentrated in vacuo, azeotroped 4× with EtOH/MeOH, and dried under vacuum at 45 °C for 18 h to give 0.138 g (64%) of product as a hygroscopic solid. ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH$_3$-NH), 2.95 (m, 2H, NCH$_2$CH$_2$O), 3.60−3.75 (m, 6H, H$_2$NCH$_2$-CH=CHCH$_2$NCH$_2$CH$_2$O), 5.75 (m, 4H, CH=CH, OCH$_2$-Ad), 7.20 (bs, 2H, NH$_2$), 8.15 (s, 1H, Ad-H8), 8.30 (s, 1H, Ad-H2). Anal. (C$_{13}$H$_{21}$N$_7$O·H$_2$SO$_4$·CH$_3$OH·H$_2$O) C, H, N, S.

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