Potential Inhibitors of S-Adenosylmethionine-Dependent Methyltransferases. 11. Molecular Dissections of Neplanocin A as Potential Inhibitors of S-Adenosylhomocysteine Hydrolase

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A series of 9-(hydroxyalkenyl)purines (adenines and 3-deazaadenines), which are analogues of neplanocin A, were synthesized. The analogues were tested as inhibitors of bovine liver and murine L929 cell S-adenosyhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1) and as inhibitors of vaccinia virus replication in murine L929 cells. Compounds **1b**, **2a**, **2b**, **4a**, **4b**, **7**, **9a**, and **9b** showed the best inhibitory effects toward bovine liver AdoHcy hydrolase, with compound **4b** being the most potent. The compounds that were shown to be the most potent inhibitors of the bovine liver AdoHcy hydrolase all contained an allylic hydroxyl group in the cis position to the adenine or the 3-deazaadenine rings. It was concluded that the cis arrangement of the allylic hydroxyl groups in these acyclic compounds represented the minimum structural requirement of the trihydroxycyclopentenyl ring of neplanocin A to show inhibitory effects against AdoHcy hydrolase. The antiviral effects of these acyclic analogues were significantly less than neplanocin A; however, there appears to be a correlation between the antiviral activity and the inhibition of AdoHcy hydrolase for compounds **2a**, **2b**, **4a**, **4b**, and **7**. Analogue **4b**, which exhibited the best antiviral activity (IC_{50} = 70 μM) in this acyclic series, is substantially less potent than neplanocin A (IC_{50} = 0.08 μM) as an antiviral agent.

S-Adenosylhomocysteine (AdoHcy) hydrolase has emerged as a potential target for the development of chemotherapeutic agents (i.e. antiviral drugs).[1,2] AdoHcy hydrolase catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine through a mechanism involving the oxidation of the 3′-hydroxyl group of the substrate with concomitant reduction of enzyme bound NAD^+ to NADH.[3-5] Inhibition of AdoHcy hydrolase causes the intracellular accumulation of AdoHcy, elevating the AdoHcy/S-adenosylmethionine (AdoMet) ratio and subsequently inhibiting cellular AdoMet-dependent methyltransferases.[1,2,6] Various inhibitors of AdoHcy hydrolase have been identified, including 3-deazaadenosine,[7] aristeromycin,[8] adenosine dialdehyde,[9] 3-deazaaristeromycin,[10] 9-(hydroxyalkyl)adenine analogues [i.e. (S)-9-(2,3-dihydroxypropyl)adenine[11] and eritadenine[12]], and neplanocin A.[13] Neplanocin A, one of the most potent inhibitors of AdoHcy hydrolase, has also been shown to have antitumor activity against murine leukemia L1210[14] and broad-spectrum antiviral activity.[13,15]

On the basis of the reported inhibitory activity toward AdoHcy hydrolase by neplanocin A[13] and the 9-(hydroxyalkyl)adenines,[11,12] we undertook the synthesis of the acyclic 9-(alkenyl)adenine analogues **1a**, **2a**, **3a**, **4a**, **5–8**, **9a**, and **10** + **11** (Chart I). Since molecular dissected analogues of 3-deazaaristeromycin exhibited some inhibitory activity toward AdoHcy hydrolase,[16] we also report here the synthesis of analogues **1b**, **2b**, **3b**, **4b**, and **9b** (Chart I) of neplanocin A containing the 3-deazaadenine base. These compounds were evaluated for their inhibitory effects toward the bovine liver and the murine L929 cell AdoHcy hydrolases. The data obtained in these studies were used to determine the minimum structural features of neplanocin A's cyclopentenyl ring necessary for binding to AdoHcy hydrolase. Compounds **1–11** (Chart I) were also tested as inhibitors of vaccinia virus replication in murine L929 cells in an attempt to further extend the correlation reported by De Clercq and Cools between the effects on AdoHcy hydrolase and antiviral activity.[17]

Results and Discussion

Chemistry. Shown in Scheme I is the synthetic route to compound **12a**, which was used as an intermediate to obtain the acetylenic compound **1a**, the Z allylic alcohol **2a** and the E allylic alcohol **3a**. Compound **12a** was synthesized from **13**[18] by nucleophilic displacement of the bromide with the sodium salt of adenine (Scheme I). Compound **12a** was then debenzoylated with methanol saturated with ammonia, giving compound **1a** in 81% yield. Catalytic hydrogenation of compound **1a** with Lindlar's catalyst gave the Z allylic alcohol **2a** with no detectable amounts of the E isomer. It is well-known that propargylic alcohols can be reduced to E allylic alcohols with high stereospecificity with lithium aluminum hydride (LAH).[19,20] Compound **12a**, with the masked alcohol, was

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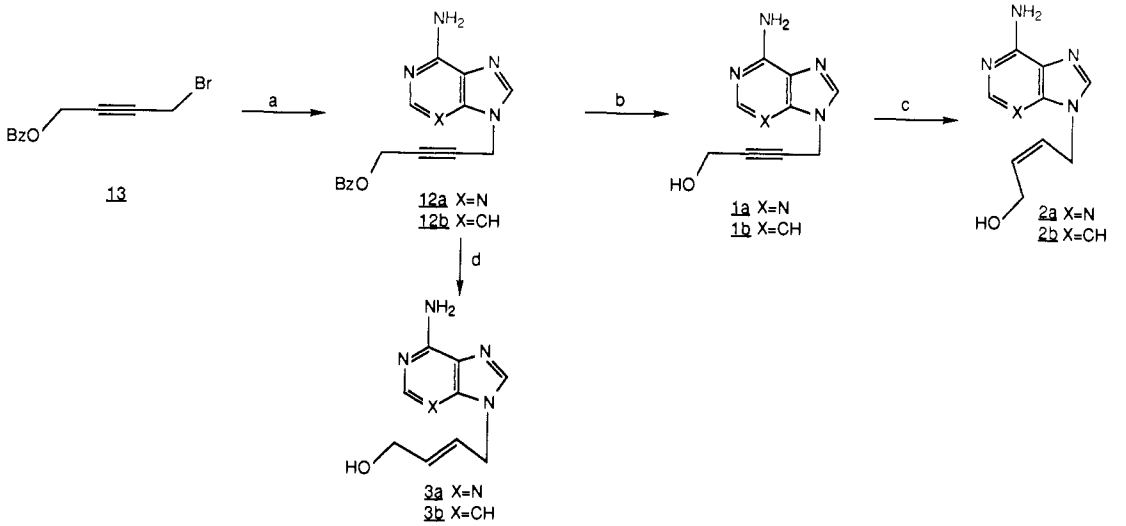
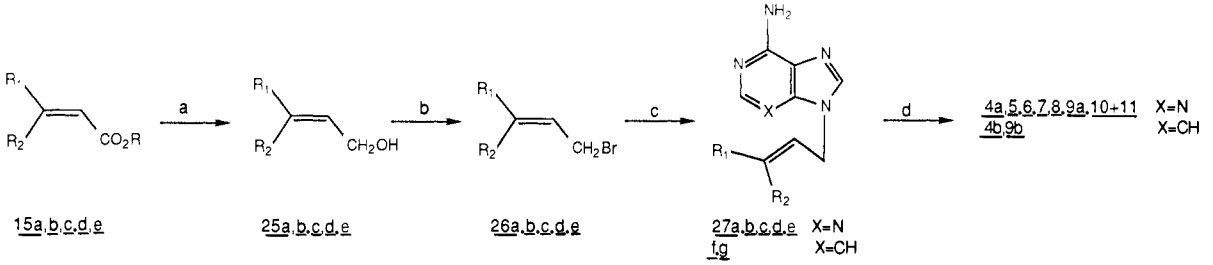
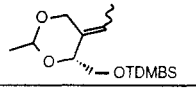
Scheme I[a]



[a] (a) NaH, DMF, adenine, or 3-deazaadenine; (b) NH_3, MeOH; (c) Lindlar, H_2, MeOH; (d) LAH, THF.

Scheme II. General Synthetic Scheme[a]



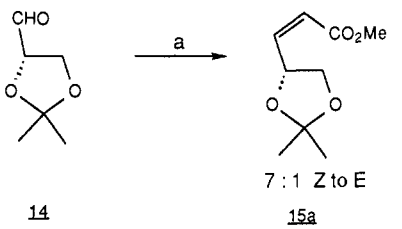
Compound	R_1	R_2
15a,25a,26a,27a,27f	H	
15b,25b,26b,27b		H
15c,25c,26c,27c	CH_3 or CH_2OTHP	CH_3 or CH_2OTHP
15d,25d,26d,27d,27g	$CH_2OCH_2OCH_2Ph$	$CH_2OCH_2OCH_2Ph$
15e,25e,26e,27e		 OTDMBS

[a] (a) DIBAH, CH_2Cl_2, -78 °C; (b) NBS, PPh_3, CH_2Cl_2; (c) NaH, DMF, adenine or 3-deazaadenine; (d) H_3O^+.

dissolved in tetrahydrofuran and reduced with LAH to afford the desired compound **3a** in 75% yield.

The general strategy for the synthesis of compounds **4a**, **4b**, **5**, **7**, **8**, **9a**, **9b**, and **10** + **11** is shown in Scheme II. This general scheme converts an α,β-unsaturated ester to the desired compounds **4a**, **4b**, **5**, **7**, **8**, **9a**, **9b**, and **10** + **11**. The esters **15a–e** (Schemes III–VII) were reduced with diisobutylaluminum hydride (DIBAH) to the allylic alcohols **25a–e**. The allylic alcohols were converted to the allylic bromides **26a–e** with *N*-bromosuccimide and triphenylphosphine, which were then nucleophilically displaced with the sodium salts of adenine or 3-deazaadenine to give the

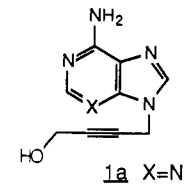
Scheme III[a]



[a] (a) $Ph_3P{=}CHCO_2Me$, MeOH, 0 °C.

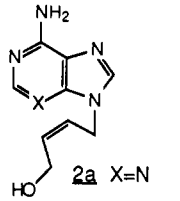
(20) Corey, E. J.; Katzenellenbogen, J. A.; Posner, G. H. *J. Am. Chem. Soc.* **1967**, *89*, 4245.

Chart I



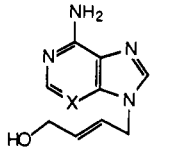
1a X=N

1b X=CH



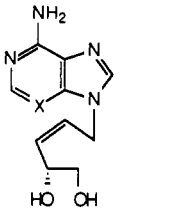
2a X=N

2b X=CH



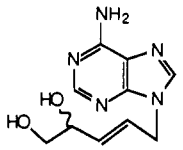
3a X=N

3b X=CH

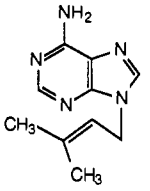


4a X=N

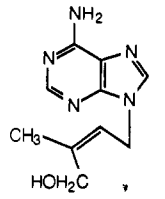
4b X=CH



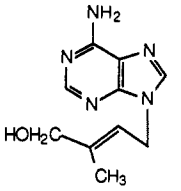
5



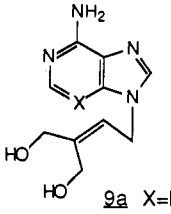
6



7

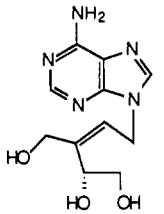


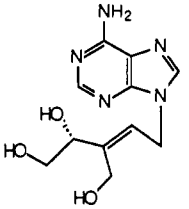
8



9a X=N

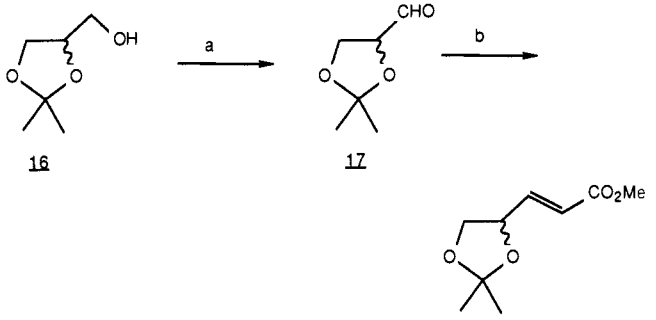
9b X=CH





10+11

Scheme IV[a]

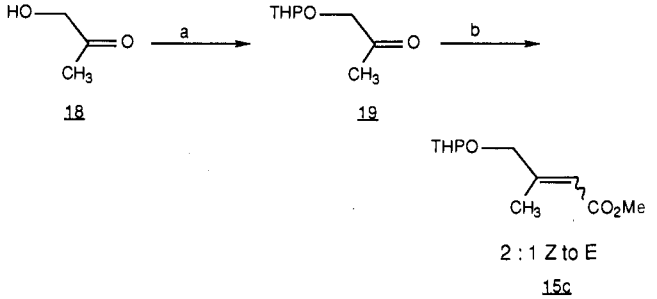


16 17 15b

17:3 E to Z

[a] (a) DMSO, oxalyl chloride, Et_3N, CH_2Cl_2, −20 °C; $(MeO)_2POCH_2CO_2Me$, NaH, THF.

Scheme V[a]



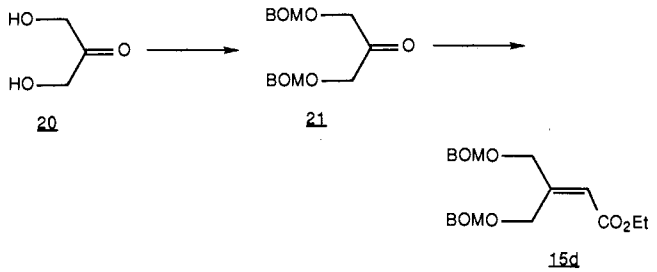
18 19

2:1 Z to E

15c

[a] (a) DHP, CH_2Cl_2, TsOH; (b) $(MeO)_2POCH_2CO_2Me$, NaH, THF.

Scheme VI[a]



20 21

15d

[a] (a) BOMCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2; (b) $(EtO)_2POCH_2CO_2Et$, NaH, THF.

protected compounds **27a–g**. These compounds were then deprotected to give the desired compounds **4a**, **4b**, **5**, **7**, **8**, **9a**, **9b**, and **10** + **11**. Compound **6** (Chart I) was synthesized from prenyl bromide by using a previously reported synthesis.[21]

The esters **15a–e** (Scheme II) were obtained with the appropriate aldehyde or ketone as the starting materials and reacting them with a phosphorus ylide according to Schemes III–VII. The ester **15a** (Scheme III) was obtained by reacting (*S*)-2,3-*O*-isopropylideneglyceraldehyde **14**[22] with methyl (triphenylphosphoranylidene)acetate, after which the ^1H NMR spectra of **15a** showed there was a 7:1 ratio of *Z* and *E* isomers. Minami et al.[23] and Tatsuki et al.[24] observed this same *Z*:*E* ratio when (*R*)-2,3-*O*-isopropylideneglyceraldehyde was used in this reaction. Compound **15a** was then converted to compounds **4a** and **4b** (Scheme II). Compound **15b** was synthesized from (*R*,*S*)-2,3-*O*-isopropylideneglyceraldehyde **17**, which was

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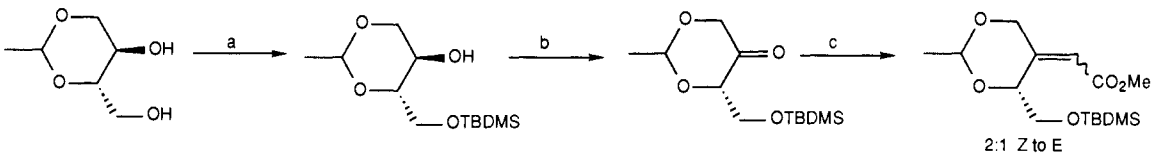
Scheme VII[a]



22 23 24 15e

[a] (a) TBDMSiCl, DMAP, Et_3N, CH_2Cl_2; (b) CrO_3, Pyr, CH_2Cl_2; (c) $(MeO)_2POCH_2CO_2Me$, NaH, THF.

obtained by the oxidation of solketal **16** (Scheme IV). The aldehyde **17** was reacted with trimethyl phosphonoacetate, which gave the ester **15b** in a 17:3 *E*:*Z* ratio. Due to the poor biological activity of compound **5**, no attempt was made to synthesize this compound in optically active form. The final compounds **4a**, **4b**, and **5** were isolated in isomerically pure form. The synthesis of **15c** started with the commercially available acetol **18**, which was protected as the tetrapyranyl ether **19** (Scheme V). The reaction of **19** with trimethyl phosphonoacetate gave a 2:1 mixture of *E* and *Z* isomers of compound **15c**, which was converted to **27c** (Scheme II). The hydrolysis of **27c** (Scheme II) gave a mixture of *E* and *Z* isomers, which were separated by HPLC with a reverse-phase column to give compounds **7** and **8**. The structural assignments of the *Z* and *E* isomers were determined by ^1H NMR. Compound **15d** (Scheme VI) was synthesized by protecting dihydroxyacetone **20** with benzyl chloromethyl ether to give compound **21**, which was converted to the ester **15d** and the final compounds **9a** and **9b** (Scheme II). The ester **15e** (Scheme VII) was obtained by the selective protection of 1,3-*O*-ethylidene-L-erythritol (**22**)[25] with *tert*-butyldimethylsilyl chloride and DMAP to give **23**, which was followed by the oxidation of **23** with chromium trioxide–pyridine to afford compound **24**. Compound **24** was reacted with trimethyl phosphonoacetate to give **15e** as an isomeric mixture. ^1H NMR showed there was a 2:1 *Z*:*E* isomeric ratio. Compounds **10** and **11** (Scheme II) were synthesized as a mixture of *Z* and *E* isomers, and no attempt was made to separate the isomers due to their poor biological activity.

It has been demonstrated by Tseng and Marquez[26] that 4-chloroimidazo[4,5-*c*]pyridine could be used to nucleophilically displace tosylates, giving the desired 1-substituted adducts with only small amounts of the 3-substituted compounds, which could be easily removed by chromatography. We found that 4-aminoimidazo[4,5-*c*]pyridine (3-deazaadenine)[27] could be used instead of 4-chloroimidazo[4,5-*c*]pyridine to displace the bromides **13**, **26a**, and **26d**. Compounds **1b**, **2b**, and **3b** (Scheme I) were synthesized from compound **12b** in the same manner as compounds **1a**, **2a**, and **3a**. Compounds **4b** and **9b** were synthesized from **26a** and **26d** according to the general procedure given in Scheme II.

Biological Results and Discussion

Inhibitory Effects on AdoHcy Hydrolase. We have previously shown that neplanocin A is an irreversible inhibitor of bovine liver AdoHcy hydrolase producing inactivation through a mechanism involving the oxidation of the 3′-hydroxyl group of the substrate with simultaneous reduction of the enzyme bound NAD^+ to NADH.[13,28] This type of inhibition could be classified as an "enzyme specific cofactor depletion mechanism". Since a number of acyclic 9-(hydroxyalkyl)adenines have shown inhibitory activity toward AdoHcy hydrolase,[11,29] we synthesized several 9-(alkenyl)adenines analogues of neplanocin A. The double bond in the acyclic analogues **1–11** limits the conformational freedom of these compounds, which should be helpful in determining what functional groups of the cyclopentenyl ring of neplanocin A are needed for its biological activity.

The effects of compounds **1–11** on bovine liver AdoHcy hydrolase were determined by using the coupled assay procedure of Richards et al.[30] Since this procedure involves the conversion of [^3H]adenosine to [^3H]inosine catalyzed by calf intestine adenosine deaminase, it was necessary to determined whether these compounds (**1–11**) had any inhibitory effects on adenosine deaminase. By use of the method of Pull and McIlwain,[31] it was shown that none of the acyclic analogues **1–11** had any significant inhibitory effects against the calf intestinal adenosine deaminase at the concentrations used in the AdoHcy hydrolase assay (data not shown). When these acyclic analogues of neplanocin A were evaluated as inhibitors of bovine liver AdoHcy hydrolase, compounds **1b**, **2a**, **2b**, **4a**, **4b**, **7**, **9a**, and **9b** were the most potent inhibitors causing time-dependent inactivation of the enzyme (Table I). The "apparent" K_i values for these analogues, which ranged from 90 to 200 nM, were determined with double-reciprocal plots of the observed rate constants versus inhibitor concentrations in a manner similar to that recently reported for neplanocin A.[32] When their inhibitory activities are compared to the inhibitory activity of neplanocin A (K_i = 3.8 nM),[32] these acyclic analogues are significantly less potent as inhibitors of bovine liver AdoHcy hydrolase. Compounds **2a**, **2b**, **4a**, **4b**, **7**, **9a**, and **9b** were also evaluated as inhibitors of murine L929 cell AdoHcy hydrolase by treating cells for 1 h with the potential inhibitors and then measuring the residual enzyme activity in cell lysates. Inhibitory effects shown in Table I are expressed as IC_{50} values (concentrations needed to produce 50% inhibition of cell AdoHcy hydrolase). A good correlation was observed between the K_i values observed with the bovine liver enzyme and the IC_{50} values observed with the L929 cell enzyme for analogues **1b**, **2a**, **2b**, **4a**, **4b**, **7**, **9a**, and **9b**. It should be noted that the synthesis and biological activity of analogue **9a** was recently reported by Haines et al.,[33] and they reported that this analogue did not elevate AdoHcy levels in HT29 cells.

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Table I. Inhibition of Bovine Liver and L929 AdoHcy Hydrolase and Vaccinia Virus Replication by the Acyclic Neplanocin A Analogues

compd	time-dependent inactivation	bovine liver AdoHcy hydrolase: K_i,[a] nM	bovine liver AdoHcy hydrolase: IC_{50},[b] μM	AdoHcy hydrolase L929 lysate: IC_{50},[c] μM	vaccina virus plaque inhibition: IC_{50},[d] μM
1a	no	na[e]	1.0	nd	>320
2a	yes	125	na	145	108
3a	yes	330	na	230	109
4a	yes	200	na	100	100
5	no	na	0.95	nd	250
6	no	na	0.93	nd	60
7	yes	170	na	200	220
8	yes	500	na	nd	300
9a	yes	110	na	65	700
10 + 11	yes	>1000	na	nd	170
1b	yes	160	na	200	300
2b	yes	200	na	200	300
3b	no	na	1.23	nd	40
4b	yes	90	na	61	70
9b	yes	166	na	110	500
NcpA	yes	3.8	na	0.05	0.08

[a] AdoHcy hydrolase activity was determined by using the procedure of Richards et al.,[30] which involves measuring the hydrolysis of [2,2-^{3}H]AdoHcy to [2,8-^{3}H]adenosine and homocysteine. The assay includes adenosine deaminase, which converts [2,8-^{3}H]adenosine to [2,8-^{3}H]inosine, and the [2,8-^{3}H]inosine is measured by scintillation spectrometry after separation on SP-Sephadex-C-25. The reaction is started by adding the AdoHcy hydrolase to a solution of inhibitor (40, 80, and 120 nM for compounds **1b**, **2a**, **2b**, **3a**, **4a**, **4b**, **7**, **8**, **9a**, and **9b**; 0.5, 1.0, and 1.5 μM for compound **10 + 11**) in phosphate buffer. The reaction was stopped at the indicated times (2, 4, 6, and 8 min for compounds **1b**, **2a**, **2b**, **3a**, **4a**, **4b**, **7**, **8**, **9a**, and **9b**; 5, 10, 15, and 20 min for compound **10 + 11**) with 5 N formic acid. For the compounds showing time-dependent inactivcation the apparent K_i values were determined with double-reciprocal plots of the observed rate constants versus inhibitor concentration. [b] The IC_{50} values for comounds **1a**, **3b**, **5**, and **6** were calculated by incubating them with AdoHcy hydrolase for 15 min at 37 °C at different concentrations (0.5, 1.0, and 1.5 μM) and then assayed for residual activity by the method of Richard et al.[30] [c] AdoHcy hydrolase activity from murine L929 cells was determined by treating the cells for 1 h with inhibitor, after which the cells were harvested and the AdoHcy hydrolase activity remaining in the lysate was determined by using the procedure of Richards et al.[30] [d] Antiviral activity was measured with monolayer cultures of mouse L929 cells for virus plaque assays and were treated as described in the text. See the Experimental Section for details. [e] na (not applicable); nd (not determined).

Compounds **2a**, **2b**, **4a**, **4b**, **7**, **9a**, and **9b** all contain an allylic hydroxyl group in a cis orientation to the adenine base as a common structural feature. These cis allylic hydroxyl groups can be viewed as the equivalent of the 3′-position of neplanocin A's carbocyclic ring. The oxidation of the 3′-position of neplanocin A has been shown to be involved in the irreversible inactivation of bovine liver AdoHcy hydrolase.[28] It appears that acyclic analogues containing a cis allylic hydroxyl group, which mimics the 3′-position of neplanocin A's carbocyclic ring, represent the minimal structural requirement necessary for neplanocin A's inhibitory activity. By studying several aliphatic acyclic analogues, Holy et al.[29] have concluded that only compounds that mimic the configuration of the ribose ring will show significant inhibition toward AdoHcy hydrolase. However, our data suggest that only a 3′-hydroxyl group locked in the correct juxtaposition to the adenine ring is required for inhibitory activity against AdoHcy hydrolase, not the 2′,3′ vicinal diol group as suggested by Holy et al.[29] and Haines et al.[33]

Compounds **3a** and **8**, which have a trans allylic hydroxy groups, also produced time-dependent inactivation of AdoHcy hydrolase, but they are significantly less active than the corresponding compounds with cis allylic hydroxy groups (**2a** and **7** in Table I). The mixture of compounds **10 + 11**, which have both trans and cis allylic hydroxy groups, produced time-dependent inactivation of bovine liver AdoHcy hydrolase only at very high concentrations (K_i = >1000 nM). This poor activity observed with this mixture of compounds (**10 + 11**) was not unexpected since a large number of acyclic polyhydroxylated adenine nucleosides have shown no inhibitory activity against AdoHcy hydrolase.[16,29]

Interesting differences were observed between the AdoHcy hydrolase inhibitory activities of the two acetylenic compounds **1a** and **1b**. Analogue **1a** was a poor inhibitor (IC_{50} = 1 μM) and did not produce time-dependent inactivation of bovine liver AdoHcy hydrolase, whereas analogue **1b** was a good inhibitor (K_i = 160 nM) and produced time-dependent inactivation of the enzyme. Compounds **3b**, **5**, and **6** were also poor inhibitors (IC_{50} > 1 μM) of the bovine liver AdoHcy hydrolase and did not produce time-dependent enzyme inactivation.

Glazer et al.[34,35] recently reported that 3-deazaneplanocin A (C3-NpcA) was a more potent inhibitor of hamster liver AdoHcy hydrolase and was significantly less cytotoxic than neplanocin A. C3-NpcA was not a substrate for adenosine kinase, nor was it deaminated by adenosine deaminase as is the case for neplanocin A. It is believed the reduced cytotoxicity of C3-NpcA is due to the fact it is not converted by adenosine kinase to nucleotide metabolites. On the basis of these results and on the previous data reported by our laboratory on acyclic 3-deazaaristeromycin analogues,[16] we attempted to improve the inhibitory effects of several of these olefinic compounds by synthesizing analogues containing the 3-deazaadenine base (compounds **1b**, **2b**, **3b**, **4b**, and **9b**). All of the 3-deazaadenine analogues (**1b**, **2b**, **4b**, and **9b**), except for compound **3b**, had similar inhibitory activity against both the bovine liver and the murine L929 cell AdoHcy hydrolases (Table I).

Antiviral Activity. Previous work from this laboratory[13] and other laboratories[15] has shown neplanocin A to be a potent, broad-spectrum antiviral agent. De Clercq and Cools[17] recently demonstrated that the inhibition of AdoHcy hydrolase by adenosine analogues (including neplanocin A) could be correlated with their antiviral activity. The antiviral effects of these compounds are believed to be due to the inhibition of (guanine-7)-*N*-methyltransferase (GNMT) and (nucleoside-2′)-*O*-methyltransferase (NOMT) which results from the ele-

(34) Glazer, R. I.; Hartman, K. D.; Knode, M. C.; Richards, M. M.; Chiang, P. K.; Tseng, C. K. H.; Marquez, V. E. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 688.
(35) Glazer, R. I.; Knode, M. C.; Tseng, C. K. H.; Haines, D. R.; Marquez, V. E. *Biochem. Pharmacol.* **1986**, *35*, 4523.

vated intracellular concentrations of AdoHcy resulting from the inhibition of AdoHcy hydrolase.[36,37] These methyltransferase are involved in critical methylations of the 5'-cap structure of viral mRNA. It has been shown that the methylated cap structure enhances translational efficiency by facilitating the binding of viral mRNA to ribosomes.[38-40] Both GNMT and NOMT have been isolated from vaccinia virus and have been shown to be sensitive to inhibition by AdoHcy.[40-42]

Since several of the acyclic analogues synthesized in this study were shown to inhibit murine L929 cell AdoHcy hydrolase (Table I), we tested these analogues for their ability to inhibit vaccinia virus replication in the same cell line. The antivaccinia virus effects were determined by measuring the inhibition of plaque formation 48 h after infection of murine L929 cell monolayers with virus, and the values are expressed as IC_{50} values (concentration needed to produce 50% inhibition of viral plaque formation)(Table I). Compounds **1b**, **2a**, **2b**, **4a**, **4b**, and **7** appear to exhibit a good correlation between the inhibition of L929 cell AdoHcy hydrolase and the inhibition of vaccinia virus replication. It should be noted, however, that the concentrations of the acyclic analogues **1b**, **2a**, **2b**, **4a**, **4b**, and **7** needed to produce 50% inhibition of viral replication are 875–3750 times greater than the IC_{50} value (0.08 μM) for neplanocin A. Differences of similar magnitude were observed between the concentrations of the acyclic analogues needed to produce 50% inhibition of L929 cell AdoHcy hydrolase (IC_{50} = 61–200 μM) and IC_{50} (0.05 μM) for neplanocin A.

The exceptions to the correlation between the ability to inhibit L929 cell AdoHcy hydrolase and the inhibition of vaccinia virus replication were analogues **9a** and **9b**. Analogues **9a** and **9b** both showed moderate activity as inhibitors of cellular AdoHcy hydrolase [IC_{50} = 65 μM (**9a**) and 110 μM (**9b**)], but both analogues were essentially inactive as inhibitors of viral replication [IC_{50} = 700 μM (**9a**) and 500 μM (**9b**)]. Several acyclic analogues (**3a**, **3b**, and **6**) that were poor inhibitors of AdoHcy hydrolase showed moderate antiviral activity (IC_{50} = 40–108 μM). Compound **3a** was reported previously to produce cytotoxicity.[43,44] Both **3a** and **3b** were also found in this study to be cytotoxic to murine L929, which could explain the apparent antiviral effects.

Experimental Section

Biological Procedures. The acyclic analogues 1–11 of neplanocin A were tested for their ability to inhibit bovine liver and murine L929 cell AdoHcy hydrolases, adenosine deaminase, and vaccinia virus replication in murine L-929 cells.

AdoHcy Hydrolase Assay. AdoHcy hydrolase activity was determined in the direction of hydrolysis with [2,8-^{3}H]AdoHcy (40 μCi/mmol) according to the procedures of Richards et al.[30] A typical incubation mixture (500 μL) contained 150 mM potassium phosphate, pH 7.6, an appropriate concentration of inhibitor, and 1.0 mM EDTA. The reaction was started by the addition of purified bovine liver AdoHcy hydrolase.[45] After the indicated times, [2,8-^{3}H]AdoHcy (100 μM, 7.1 mCi/mmol) and 4 units of intestinal adenosine deaminase (A unit of adenosine deaminase is defined by the supplier as the following: 1 unit will deaminate 1.0 μmol of adenosine to inosine per minute at pH 7.5 at 25 °C) were added, and the reaction was incubated for another 5 min. The reaction was stopped by the addition of 100 μL of 5 N formic acid, and the reaction mixture was then poured onto a column (1.2 × 4 cm) of SP-Sephadex-C-25, which was previously equilibrated with 0.1 N formic acid. Each column was first rinsed with 0.5 mL of 0.1 N formic acid and [2,8-^{3}H]inosine; the product of the deamination of [2,8-^{3}H]adenosine formed by the hydrolysis of [2,8-^{3}H]AdoHcy was subsequently eluted from the column by addition of 8 mL of 0.1 N formic acid. A 1-mL aliquot of the eluate was added to 10 mL of 3a70 scintillation fluid and the radioactivity determined. The results were corrected with the blank which contained everything except AdoHcy hydrolase.

Murine L929 AdoHcy Hydrolase Assay. Confluent murine L929 cells were grown (6 × 10^6 cells/100-mm dish) in waymouth MB medium containing 2% calf serum. The cells were then incubated with the drug (1, 50, 100, and 200 μM) for 1 h at 37 °C. The cells were then harvested by aspirating the culture medium followed by washing with phosphate-buffered saline (PBSA); then the cells were treated with a 0.1% trypsin solution for 1 min. The trypsin solution was removed by aspiration, and the plates were left at room temperature for 5 min. The cells were suspended in 0.6 mL of cold PBSA and were transfered to an Eppendorf tube. The cells were diluted with an additional 0.6 mL of PBSA and centrifuged for 1 min at 12 500 rpm, and the supernatant was removed. The pellet was resuspended in 0.5 mL of hypotonic buffer and vortexed for 1 min. The tubes were immersed in a dry ice–acetone bath and stored at −78 °C. The Eppendorf tubes were thawed, and the homogeneous suspension was centrifuged for 1 min. Then 450 μL of the supernatant was diluted with 450 μL of buffer and assayed for the AdoHcy hydrolase activity.

Adenosine Deaminase Assay. Adenosine deaminase activity was assayed by the method of Pull and McIlwain[31] and modified accordingly. The reaction mixture (total volume 500 μL) contained 0.3 μM [2,8-^{3}H]adenosine (30 Ci/pmmol), 0.1 unit of adenosine deaminase, and 150 μM potassium phosphate buffer, pH 7.6, containing 50 μg/mL bovine serum albumin. The reaction was incubated at 25 °C for 5 min and terminated by the addition of 100 μL of 0.5 N formic acid. The mixture was layered onto a SP-Sephadex column and eluted with 8 mL of 0.1 N formic acid. [2,8-^{3}H]Inosine was quantified by a liquid scintillation counter. The assays were conducted so that only 5–20% of the adenosine was deaminated. In the inactivation process, adenosine deaminase was incubated with 0.6 μM of the respective inhibitor at 25 °C for 10 min, and the residual enzyme activity was determined as described above.

Vaccinia Plaque Assay. Experimental cultures for antivaccinia virus assays were plated in multiculture (Costar) dishes having 24 wells (13-mm diameter). Nearly confluent cell monolayers were infected with vaccinia virus at about 200 plaque forming units per well. After a 60-min adsorption period, the viral inoculum was diluted by the addition of KU-1 culture medium and then completely removed by aspiration. Immediately after infection, each infected cell monolayer was overlayed with 400 μL of KU-1 medium containing 0.13% methylcellulose and 1% calf serum, to which was added with mixing 100 μL of KU-1 medium containing test compound in 0.5 log dilutions of 10, 32, 100, and 320 μM. After incubation for 48 h at 37 °C, cultures were washed with phosphate-buffered saline (PBSA) and stained with 0.1% crystal violet. The plaque number was then counted under a microscope. In these experiments, the antiviral activities of the compounds against vaccinia virus replication were assayed in duplicate and 50% inhibitory concentration (IC 50) values were determined from concentration-dependent plaque reduction curve.

Synthesis. Melting points were determined on a Fisher-Johns stage melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian FT-80A (80 MHz for proton nuclei)

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spectrophotometer. Chemical shifts are reported in ppm from the internal standard tetramethylsilane (TMS), $\delta_{TMS} = 0.00$. Mass spectra was recorded on a Ribermag R10-10 quadrupole spectrometer. Peak matching was performed by a Varian MAT CH5 magnetic deflection mass spectrometer. The optical rotations were determined on a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed on an F and M Model 185 C, H, N analyzer in the Department of Medicinal Chemistry, University of Kansas. Ion-exchange chromatography was performed with a strong cation exchange resin Dowex 50-W (H^+ from) stock No. 50X4-200, 4% cross-linked, 100–200 dry mesh size. Thin-layer chromatography was carried out with Analtech 0.25-mm silica gel GF glass backed plates. Preparative-layer chromatography was performed with a Model 7924T chromatotron with 1, 2, and 4 mm silica gel PF-254 with $CaSO_4 \cdot {}^1/_2H_2O$ (Merck) plates. HPLC separations were carried out on a Beckman 112A HPLC modular unit using an Analtech PRP-1 reverse-phase semipreparative column.

9-[4-(Benzoyloxy)but-2-ynyl]adenine (12a). To a stirred solution of adenine (2.4 g, 18 mmol) in 50 mL of DMF was added NaH (80% in mineral oil, 0.51 g, 18 mmol), and the solution was stirred for 30 min at room temperature. Then 1-(benzoyloxy)-4-bromo-2-butyne[18] (**13**, 4.55 g, 18 mmol) was added and the reaction mixture was stirred for 6 h at room temperature. The DMF was removed by distillation, leaving a residue which was dissolved with $CHCl_3$/EtOH (19:1). The solid that did not dissolve was removed by suction filtration and the filtrate was concentrated to dryness. The product was then recrystallized from hot EtOH to yield 3.7 g (67%): mp 186–187 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.23 and 8.18 (2 s, 2 H), 8.02 and 7.95 (2 d, 2 H), 7.57 (m, 3 H), 7.25 (s, 2 H, NH_2, exchanged), 5.08 and 5.01 (2 s, 4 H); MS (D-EI, MeOH), *m/e* 307 (5), 202 (60), 77 (100). Anal. ($C_{16}H_{13}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

9-(4-Hydroxybut-2-ynyl)adenine (a). 9-[4-(Benzoyloxy)-but-2-ynyl]adenine (**12a**, 307 mg, 1 mmol) was dissolved in 20 mL of MeOH saturated with NH_3 and placed in a sealed vessel at room temperature for 24 h. The precipitate that formed was collected by filtration and dried to yield 190 mg (83%): mp 230 °C dec; ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.20 and 8.17 (2 s, 2 H), 5.05 (br s, 2 H), 4.08 (br s, 2 H); MS (D-EI, MeOH), *m/e* 203 (100), 186 (40), 135 (17). Anal. ($C_9H_9N_5O \cdot H_2O$) C, H, N.

9-[(*Z*)-4-Hydroxybut-2-enyl]adenine (2a). 9-(4-Hydroxybut-2-ynyl)adenine (**1a**, 200 mg, 0.65 mmol) was added to a solution of MeOH containing Lindlar's catalyst ($Pd/CaCO_3$, poisoned with lead) and 1 drop of quinoline. The mixture was hydrogenated for 3 h under 30 psi of H_2. The solution was filtered and concentrated to dryness to yield 180 mg (97%): mp 198–199 °C; ^{1}H NMR (DMSO-d_6) δ 8.10 and 8.05 (2 s, 2 H), 7.09 (br s, 2 H, NH_2, exchanged), 5.63 (m, 2 H), 4.77 (d, 2 H, J = 5 Hz), 4.17 (d, 2 H, J = 5 Hz); MS (D-EI, MeOH), *m/e* 205 (5), 174 (100), 135 (48), 108 (85). Anal. ($C_9H_{11}N_5O \cdot {}^1/_4H_2O$) C, H, N.

9-[(*E*)-4-Hydroxybut-2-enyl]adenine (3a). 9-[4-(Benzoyloxy)but-2-ynyl]adenine (**12a**, 400 mg, 1.3 mmol) was dissolved in 120 mL of dry THF and 4 equiv of LAH (296 mg, 5.2 mmol) was added and the solution was stirred for 6.5 h at room temperature. Then 1 mL of H_2O was added carefully and the precipitate that formed was filtered and washed with $CHCl_3$/EtOH (9:1). The filtrate was concentrated and loaded onto a 3-g column of silica gel and was eluted with $CHCl_3$/EtOH (9:1), yielding 200 mg of the compound (74%): mp 200 °C (lit.[43,44] mp 201 °C); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.14 and 8.11 (2 s, 2 H), 5.73 (m, 2 H, J = 14 Hz), 4.76 (d, 2 H, J = 5 Hz), 3.91 (d, 2 H, J = 5 Hz); MS (D-EI, MeOH), *m/e* 205 (4), 174 (100), 135 (14), 108 (30). Anal. ($C_9H_{11}N_5O \cdot {}^1/_2H_2O$) C, H, N.

4-Amino-1-[4-(benzoyloxy)but-2-ynyl]imidazo[4,5-*c*]-pyridine (12b). Compound **12b** was prepared in the same manner as **12a**, starting from **13** (1.01 g, 4.0 mmol), except 3-deazaadenine[27] was used in place of adenine to yield 0.67 g (56%): mp 164 °C; ^{1}H NMR ($CDCl_3$ + D_2O) δ 8.07 and 7.79 (2 d, 2 H), 7.88 (d, 1 H, J = 8 Hz), 7.87 (s, 1 H), 7.53 (m, 3 H), 6.81 (d, 1 H, J = 8 Hz), 4.92 (br s, 4 H); MS (D-EI, CH_2Cl_2, *m/e* 306 (24), 201 (14), 134 (8), 105 (100). Anal. ($C_{17}H_{14}N_4O \cdot H_2O$) C, H, N.

4-Amino-1-(4-hydroxybut-2-ynyl)imidazo[4,5-*c*]pyridine (1b). Compound **1b** was prepared in the same manner as **1a**, starting from **12b** (100 mg, 0.33 mmol), to yield 60 mg (98%): mp 163–164 °C; ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1 H), 7.66 (d, 1 H, J = 7 Hz), 6.79 (d, 1 H, J = 7 Hz), 6.15 (br s, 2 H, NH_2, exchanged), 5.08 (t, 2 H, J = 0.5 Hz), 4.03 (t, 2 H, J = 0.5 Hz); MS (D-EI, MeOH), *m/e* 202 (100), 174 (84), 106 (76). Anal. ($C_{10}H_{10}N_4O \cdot H_2O$) C, H, N.

4-Amino-1-[(*Z*)-4-hydroxybut-2-enyl]imidazo[4,5-*c*]-pyridine (2b). Compound **2b** was prepared in the same manner as **2a**, starting from **12b** (32 mg, 0.15 mmol), to yield 32 mg (99%): mp 125–126 °C; ^{1}H NMR (CD_3OD) δ 8.09 (s, 1 H), 7.69 (d, 1 H, J = 10 Hz), 6.91 (d, 1 H, J = 10 Hz), 5.71 (m, 2 H, J = 10 Hz, J = 6 Hz), 4.88 (d, 2 H, J = 6 Hz), 4.33 (d, 2 H, J = 6 Hz); MS (D-EI, MeOH), *m/e* 204 (46), 135 (100), 107 (72). Anal. ($C_{10}H_{12}N_4O \cdot H_2O$) C, H, N.

4-Amino-1-[(*E*)-4-hydroxybut-2-enyl]imidazo[4,5-*c*]-pyridine (3b). Compound **3b** was prepared in the same manner as **3a**, starting from **12b** (50 mg, 0.16 mmol), to yield 24 mg (75%): mp 185 °C (hydroscopic); ^{1}H NMR (CD_3OD) δ 8.07 (s, 1 H), 7.69 (d, 1 H, J = 8 Hz), 6.86 (d, 1 H, J = 8 Hz), 5.85 (m, 2 H, J = 14 Hz, J = 5 Hz), 4.75 (d, 2 H, J = 5 Hz), 4.07 (d, 2 H, J = 5 Hz); MS (D-EI, MeOH), *m/e* 204.10103 (calcd), 204.10093 (found) (60), 173 (14), 134 (100), 107 (58).

(*R*,*S*)-1,2-*O*-Isopropylideneglyceraldehyde (17). To a solution of oxalyl chloride (10.15 g, 80 mmol) in 150 mL of CH_2Cl_2 at −78 °C was added dropwise DMSO (6.25 g, 80 mmol) in 30 mL of CH_2Cl_2 over a 3-min period. Solketal (**16**) (10 g, 75.6 mmol) in 50 mL of CH_2Cl_2 was then added over a 15-min period and the solution was stirred for 20 min, allowing the mixture to warm to 20–30 °C. Triethylamine (8.1 g, 160 mmol) was added and the mixture was stirred for 5 min after which hexane was added to precipitate $Et_3N \cdot HCl$. After the solution was filtered and concentrated (40 °C at 60 mmHg), a mixture of ether/cyclohexane (1:1) was added to remove more of the hydrochloride salt; this was repeated two times. The solution was concentrated as before and distilled (45–48 °C at 15 mm Hg) to give 3.94 g (40%) of **17**, which had a identical boiling point and NMR spectra with those previously reported (lit.[46] bp 46–48 °C at 15 mmHg).

1-(Tetrahydropyranyloxy)-2-propanone (19). Acetol (**18**, 10 g, 135 mmol) and 3,4-dihydro-2*H*-pyran (22.88 g, 272 mmol) were dissolved in 50 mL of CH_2Cl_2 which was cooled to 0 °C; then a catalytic amount of *p*-toluenesulfonic acid (0.1 g) was added (very exothermic reaction) and the solution was stirred for 2 h at room temperature. The solution was neutralized with solid $NaHCO_3$ (200 mg) and extracted with H_2O and brine, and the organic layer was dried over Na_2SO_4, filtered, and concentrated to a brown liquid, which was distilled (60 °C at 0.6 mmHg) to yield 11.1 g (52%): IR (neat) 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.55 (t, 1 H), 4.18 (s, 2 H), 4.0–3.5 (m, 2 H), 2.25 (s, 3 H), 2.0–1.4 (m, 6 H); MS (D-EI, MeOH), *m/e* 158 (12), 128 (54), 101 (28), 85 (100). Anal. ($C_8H_{14}O_3$) C, H.

1,3-Bis[(benzyloxy)methoxy]-2-propanone (21). Dihydroxyacetone (**20**, 10 g, 55 mmol) was suspended in 300 mL of CH_2Cl_2 with external cooling to 0 °C, and then benzyl chloromethyl ether (43.5 g, 277.5 mmol) was added followed by diisopropylethylamine (35.9 g, 277.5 mmol) and the reaction mixture was stirred at room temperature for 24 h. The mixture was then extracted with dilute HCl, H_2O, saturated $NaHCO_3$, and brine, and the organic layer was dried over Na_2SO_4, filtered, and concentrated to a yellow liquid. The yellow liquid was passed through a column of silica gel (50 g) with CH_2Cl_2/hexane (3:1) as eluent. Attempts to purify the compound by distillation resulted in decomposition (180 °C at 0.1 mmHg). The compound was isolated in a crude yield of 29.4 g (79.3%): IR (neat) 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.23 (s, 10 H), 4.7 (s, 4 H), 4.55 (s, 4 H), 4.29 (s, 4 H); MS (DCI, CH_4), *m/e* 329 (20), 209 (12), 121 (100).

4-(*tert*-Butyldimethylsilyl)-1,3-*O*-ethylidene-L-erythritol (23). The erythritol[25] **22** (5.30 g, 35.8 mmol) was dissolved in 50 mL of $CHCl_3$ to which TBDMSiCl (5.93 g, 39 mmol), a catalytic amount of DMAP (175 mg, 1.4 mmol), and Et_3N (4.0 g, 39 mmol) were added. The solution was stirred overnight at room temperature, and then it was extracted with H_2O and brine and the organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The product was passed through a 10-g column of silica gel with CH_2Cl_2, which gave 8.91 g (95%) of compound **23**: bp 110 °C (external) at 0.1 mmHg; $[\alpha]_D$ −46.5° (*c* 2.1, MeOH);

(46) Baer, E.; Fischer, H. O. L. *J. Am. Chem. Soc.* **1939**, *61*, 751.

^{1}H NMR ($CDCl_3$) δ 5.11 (q, 1 H, J = 5 Hz), 4.1–3.21 (m, 6 H), 1.26 (d, 3 H, J = 5 Hz), 0.95 (s, 9 H), 0.06 (s, 6 H); MS (D-CI, NH_3), m/e 263 (7), 207 (24), 117 (68), 75 (100). Anal. ($C_{12}H_{26}O_4Si$) C, H.

4-(*tert*-Butyldimethylsilyl)-1,3-*O*-ethylidene-L-*glycero*-tetrulose (24). To a solution of pyridine (17.8 g, 226 mmol) in 250 mL of CH_2Cl_2 was added CrO_3 (11.42 g, 114 mmol), and then the solution was allowed to stir for 30 min. Compound **23** (5.0 g, 19 mmol) was added in one portion, and the solution was then stirred for 30 min at room temperature. The solution was then decanted and the tar was triturated with Et_2O and decanted (repeated several times). The combined organics were filtered through Celite and concentrated to approximately 15 mL; then more Et_2O was added and the precipitate was removed by filtration. The filtrate was concentrated and passed through a column of silica gel (10 g) with Et_2O as eluent. The filtrate was concentrated to give 3.96 g (75%) of compound **24**, which was used immediately without further purification: IR (neat) 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.01 (q, 1 H, J = 6 Hz), 4.21 (br s, 3 H), 3.91 (dd, 2 H, J = 4 Hz), 1.39 (d, 3 H, J = 6 Hz), 0.90 (s, 9 H), 0.06 (s, 6 H).

(*R*)-Methyl (*Z*)-4,5-(Isopropylidenedioxy)-2-pentenoate (15a). The procedure used by Minani et al.[23] for the synthesis of the *S* enantiomer of **15a** was employed here for the preparation of the *R* enantiomer. Compound 14[22] (3.3 g, 25.38 mmol) was dissolved in 100 mL of methanol and methyl (triphenylphosphoranylidene)acetate (8.5 g, 25.3 mmol) was added; then the mixture was placed in the refrigerator for 48 h. The mixture was concentrated and dry Et_2O was added to precipitate out triphenylphosphine oxide, which was removed by filtration. The liquid was distilled (105–108 °C at 4 mmHg) to give 3.13 g (70%) of the desired compound **15a**: $[\alpha]_D$ −115° (*c* 4.0, $CHCl_3$) (lit. for *S* enantiomer,[23,24] $[\alpha]_D$ +129° (*c* 2.63, $CHCl_3$)); IR (neat) 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.38 (dd, 1 H, J = 10 Hz), 5.84 (d, 1 H, J = 10 Hz), 5.50 (dq, 1 H, J = 7 Hz, J = 1 Hz), 4.39 (dd, 1 H, J_{ab} = 7 Hz), 3.75 (s, 3 H), 3.69 (dd, 1 H, J_{ab} = 7 Hz), 1.41 (2 s, 6 H); MS (D-CI, NH_3), m/e 187 (27), 145 (26), 129 (100), 111 (26). Anal. ($C_9H_{14}O_4$) C, H.

(*R,S*)-Methyl *trans*-4,5-(Isopropylidenedioxy)-2-pentenoate (15b). To a slurry of NaH (80%, 0.87 g, 30.3 mmol) in 250 mL of THF was added dropwise trimethyl phosphonoacetate (5.52 g, 30.3 mmol). After 1 h of vigorous stirring, compound **17** (3.94 g, 30.3 mmol) was added in one portion. The reaction was allowed to proceed for 6 h, after which H_2O was added, and the mixture was extracted with 200 mL of Et_2O (2×), and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The resulting liquid was distilled (bp 112–115 °C at 10 mmHg) to give 4.13 g (75%) of compound **15b**: IR (neat) 1728 cm^{-1}; 17:3 *E*:*Z* ratio was determined by NMR; ^{1}H NMR ($CDCl_3$) δ 6.87 (dd, 1 H, J = 16 Hz), 6.07 (d, 1 H, J = 16 Hz), 4.63 (m, 1 H), 4.19 (dd, 1 H, J_{ab} = 7 Hz), 3.75 (s, 3 H), 3.70 (m, 1 H), 1.41 (2 s, 6 H); MS (D-CI, NH_3), m/e 187 (87), 171 (100), 129 (97), 98 (81). Anal. ($C_9H_{14}O_4$) C, H.

(*E,Z*)-Methyl 3-Methyl-4-(tetrahydropyranyloxy)-2-butenoate (15c). Compound **15c** was prepared in the same manner as **15b**, starting from **19** (8.0 g, 50.6 mmol), to yield 8.06 g (74.5%): bp 100–108 °C at 0.2 mmHg; IR (neat) 1718 cm^{-1}; 2:1 *E*:*Z* ratio was determined by NMR; ^{1}H NMR ($CDCl_3$) δ 5.95 (t, 1 H, (*E*) H-2, J = 1 Hz), 5.69 (t, 1 H, (*Z*) H-2, J = 1 Hz), 4.63 (2 s, 1 H), 4.11 and 3.97 (2 s, 2 H, (*E,Z*) H-1), 4.0–3.25 (m, 2 H), 3.71 and 3.6 (2 s, 3 H), 2.09 and 1.99 (2 s, 3 H, (*E,Z*) CH_3), 1.8–1.4 (m, 8 H); MS (D-EI, CH_2Cl_2), m/e 215 (15), 183 (18), 113 (63), 85 (100). Anal. ($C_{11}H_{18}O_4$) C, H.

Ethyl 4-[(benzyloxy)methoxy]-3-[[(benzyloxy)methoxy]-methyl]-2-butenoate (15d). Compound **15d** was prepared in the same manner as **15b**, starting from **21** (28.42 g, 85.0 mmol), except triethyl phosphonoacetate was used instead of trimethyl phosphonoacetate to yield 30.93 g (90%): bp >180 °C at 0.05 mmHg resulted in decomposition; IR (neat) 1714 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.25 (s, 10 H), 6.15 (s, 1 H), 5.14 (s, 4 H), 4.75 (s, 4 H), 4.68 (s, 4 H, H-4 and H-4′), 4.22 (q, 2 H), 1.32 (t, 3 H); MS (D-CI, NH_3), m/e 401 (7), 263 (88), 173 (41), 91 (100). An analytical sample was obtained by column chromatography (silica gel, 40 g) with CH_2Cl_2. Anal. ($C_{23}H_{28}O_6$) C, H.

(*E,Z*)-Methyl [4-[[(*tert*-Butyldimethylsilyl)oxy]-methyl]-2-methyl-1,3-dioxanylidene]acetate (15e). Compound **15e** was prepared in the same manner as **15b**, starting from **24** (3.61 g, 13.9 mmol), to yield 3.91 g (89%): $[\alpha]_D$ −9.2° (*c* 5, $CHCl_3$); IR (neat) 1722 cm^{-1}; 2:1 *Z*:*E* ratio was determined by NMR; ^{1}H NMR ($CDCl_3$) δ 5.95 (dd, 1 H, (*E*) H-2, J = 1 Hz), 5.24 (d, 1 H, (*Z*) H-2, J = 1 Hz), 4.86 (q, 1 H), 4.58 (d, 2 H, J = 2 Hz), 4.34 (d, 2 H, J = 2 Hz), 4.23 (m, 1 H, (*Z*) H-4), 3.97 (m, 1 H, (*E*) H-4), 3.85 (s, 2 H, (*Z*) H-6), 3.78 (s, 2 H, (*E*) H-6), 3.68 (s, 3 H), 1.33 (2 d, 3 H), 0.90 (s, 9 H), 0.10 (s, 6 H); MS (D-CI, NH_3), m/e 317 (10), 215 (100), 113 (43). An analytical sample obtained by distillation (140 °C at 0.1 mmHg) ($C_{15}H_{28}O_5Si$); C, H.

(*R*)-(*Z*)-3,4-(Isopropylidenedioxy)-2-penten-1-ol (25a). To a solution of compound **15a** (2.8 g, 15 mmol) in 50 mL of CH_2Cl_2 cooled to −78 °C was added dropwise 1 M DIBAH (in CH_2Cl_2, 37 mL, 37.6 mmol) over a period of 1 h. When the addition was complete, the solution was allowed to come to room temperature and stirred for 12 h. Acetone was added and the reaction mixture was stirred for 1 additional hour. To the reaction mixture was added H_2O and the precipitate that formed was filtered through a pad of Celite and the Celite pad was washed with CH_2Cl_2. The filtrate was extracted with brine, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (10 g of silica gel) with CH_2Cl_2/EtOH (9:1) to yield 2.09 g (88%) of compound **25a**: $[\alpha]_D$ −16.02° (*c* 3.6, MeOH), [lit. *S* enantiomer,[23] $[\alpha]_D$ +14.0° (*c* 4.5, MeOH)]; ^{1}H NMR ($CDCl_3$) δ 5.95–5.22 (m, 2 H), 4.83 (dq, 1 H), 4.13 (d, 2 H, J = 8 Hz), 4.06 (dd, 1 H, J_{ab} = 8 Hz), 3.55 (dd, 1 H, J_{ab} = 8 Hz), 1.42 (2 s, 6 H); MS (D-CI, NH_3), m/e 158 (16), 143 (16), 55 (100). An analytical sample was obtained by distillation (110 °C (external) at 0.1 mmHg) ($C_8H_{14}O_3$); C, H.

(*R,S*)-(*E*)-3,4-(Isopropylidenedioxy)-2-penten-1-ol (25b). Compound **25b** was prepared in the same manner as **25a**, starting from **15b** (3.63 g, 19.5 mmol), to yield 3.0 g (97%): bp 110 °C (external at 0.1 mmHg); ^{1}H NMR ($CDCl_3$) δ 6.15–5.50 (m, 2 H), 4.50 (dq, 1 H, J = 8 Hz), 4.15 (m, 3 H, J_{ab} = 8 Hz), 3.69 (dd, 1 H, J_{ab} = 8 Hz), 1.46 (2 s, 6 H); MS (D-CI, NH_3), m/e 158 (15), 143 (26), 55 (100). Anal. ($C_8H_{14}O_3$) C, H.

(*E,Z*)-3-Methyl-4-(tetrahydropyranyloxy)-2-buten-1-ol (25c). Compound **25c** was prepared in the same manner as **25a**, starting from **15c** (8.06 g, 36.6 mmol), to yield 6.2 g (95%): bp 95–102 °C at 0.2 mmHg; ^{1}H NMR ($CDCl_3$) δ 5.65 (t, 1 H, J = 7 Hz), 4.59 (br s, 1 H), 4.25–3.5 (m, 6 H), 1.71 (s, 3 H), 1.71–1.35 (m, 6 H); MS (D-EI, CH_2Cl_2), m/e 186 (7), 169 (35), 85 (100). Anal. ($C_{10}H_{18}O_3$) C, H.

4-[(Benzyloxy)methoxy]-3-[[(benzyloxy)methoxy]-methyl]-2-butenol (25d). Compound **25d** was prepared in the same manner as **25a**, starting from **15d** (29.93 g, 74.1 mmol), to yield 24.0 g (crude; 90%) (distillation at 180 °C at 0.05 mmHg resulted in decomposition); ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 10 H), 5.90 (t, 1 H, J = 7 Hz), 4.74 (2 s, 4 H), 4.60 (s, 4 H), 4.15 (m, 6 H, J = 9 Hz); MS (D-CI, CH_4), m/e 357 (5), 295 (14), 237 (100), 171 (42).

(*E,Z*)-2-[4-[(*tert*-Butyldimethylsilyl)oxy]-2-methyl-1,3-dioxanylidene]ethanol (25e). Compound **25e** was prepared in the same manner as **25a**, starting from **15e** (3.41 g, 10.8 mmol), to yield 3.05 g (98%): mp 49 °C; $[\alpha]_D$ −26.36° (*c* 2.2, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 5.69 (overlapping t, 1 H, (*E,Z*) olefin, J = 6 Hz), 4.91 (q, 1 H, J = 5 Hz), 4.70 and 4.55 (2 s, 2 H, (*E,Z*) H-6), 4.18 (d, 2 H, J = 7 Hz), 4.05 (d, 1 H, J = 6 Hz), 3.88 (dd, 2 H, J = 6 Hz), 1.35 (2 d, 3 H, (*E,Z*) CH_3), 0.95 (s, 9 H), 0.10 (s, 6 H); MS (D-CI, NH_3), m/e 289 (4), 245 (32), 187 (18), 169 (18), 75 (100). Anal. ($C_{14}H_{28}O_4Si$) C, H.

General Procedure for the Conversion of the Allylic Alcohols to Protected Acyclic Nucleosides. (*R*)-9-[(*Z*)-3,4-(Isopropylidenedioxy)pent-2-enyl]adenine (27a). The alcohol **25a** (1.0 g, 6.33 mmol) was dissolved in 40 mL of CH_2Cl_2, and triphenylphosphine (1.83 g, 6.96 mmol) was added followed by the slow addition of NBS (1.21 g, 6.77 mmol) over a 3-min period. After the addition was complete, the solution was stirred for 3 h at room temperature. The mixture was then extracted with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to a yellow liquid. The liquid was taken up in hexane/Et_2O (1:1) and the yellow precipitate that formed was removed by filtration and the filtrate was concentrated. This procedure was repeated three times and the resulting bromide **26a** was used without further purification (75–85% yields were obtained): ^{1}H NMR ($CDCl_3$) δ 5.98–5.41 (m, 2 H), 4.89 (dq, 1 H), 4.07 (dd, 1 H, J_{ab}

= 8 Hz), 3.98 (d, 2 H), 3.68 (dd, 1 H, J_{ab} = 8 Hz), 1.61 (2 s, 6 H).

The bromide **26a** was then added to a solution of sodium adenine in 30 mL of DMF. [Sodium adenine was prepared by adding NaH (80%, 0.547 g, 19 mmol) to a slurry of adenine (2.57 g, 19 mmol) in 30 mL of DMF.] The mixture was stirred at room temperature for 1–2 days, after which the DMF was removed by distillation. The residue was dissolved in CH_2Cl_2 (30–50 mL) and the undissolved material (salts) were removed by filtration. The filtrate was concentrated to dryness and the solid was dissolved in a minimum amount of CH_2Cl_2/EtOH (9:1), and then it was purified with a Model 7429T Chromatotron (4-mm silica gel plate) to yield 0.60 g (overall; 35%): mp 143–144 °C; $[\alpha]_D$ −31° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1 H), 7.27 (s, 1 H), 5.87 (br s, 2 H, NH_2, exchanged), 5.80 (m, 2 H, *J* = 9 Hz), 4.92 (m, 3 H, *J* = 7 Hz, *J* = 9 Hz), 4.18 (dd, 1 H, J_{ab} = 9 Hz), 3.64 (dd, 1 H, J_{ab} = 9 Hz), 1.44 (2 s, 6 H); MS CI, NH_3), *m/e* 276 (100), 217 (18), 136 (23). Anal. ($C_{13}H_{17}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

(*R*,*S*)-9-[(*E*)-3,4-(Isopropylidenedioxy)pent-2-enyl]adenine (27b). Compound **27b** was prepared in the same manner as **27a**, starting from **25b** (3.8 g, 24.1 mmol). ^{1}H NMR for the intermediate bromide **26b**, ($CDCl_3$) δ 6.18–5.55 (m, 2 H), 4.49 (dq, 1 H, *J* = 7 Hz), 4.03 (dd, 1 H, J_{ab} = 8 Hz), 3.80 (d, 2 H, *J* = 8 Hz), 3.62 (dd, 1 H, J_{ab} = 8 Hz), 1.40 (2 s, 6 H).

Overall yield of compound 27b: 4.86 g (53%); mp 144–145 °C; ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1 H), 7.81 (s, 1 H), 6.23 (br s, 2 H, NH_2, exchanged), 6.10–5.48 (m, 2 H), 4.84 (d, 2 H, *J* = 6 Hz), 4.5 (dq, 1 H, *J* = 8 Hz), 4.10 (dd, 1 H, J_{ab} = 8 Hz), 3.57 (dd, 1 H, J_{ab} = 8 Hz), 1.40 (2 s, 6 H); MS (CI, NH_3), *m/e* 276 (100), 179 (90), 136 (14). Anal. ($C_{13}H_{17}H_5O_2$) C, H, N.

(*E*,*Z*)-9-[3-Methyl-4-(tetrahydropyranyloxy)but-2-enyl]adenine (27c). Compound **27c** was prepared in the same manner as **27a**, starting from compound **25c** (0.9 g, 5.1 mmol) (a mixture of *E* and *Z* isomers). ^{1}H NMR for the intermediate bromide **26c**, ($CDCl_3$) δ 5.72 (t, 1 H, *J* = 6 Hz), 4.61 (br s, 1 H), 4.30–3.30 (m, 6 H, H-1), 1.83 (2 s, 3 H, (*E*,*Z*) CH_3), 1.80–1.40 (m, 6 H).

Overall yield of compound 27c: 0.59 g (50%) of an oily solid; ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1 H), 7.88 and 7.81 (s, 1 H), 6.82 (br s, 2 H, NH_2, exchanged), 5.67 and 5.55 (2 t, 1 H, (*Z*) H-2′ and (*E*) H-3′), 4.88 and 4.75 (2 d, 2 H, (*Z*) H-1′ and (*E*) H-1′, *J* = 8 Hz), 4.65 (br s, 1 H), 4.10 (s, 2 H, (*Z*) H-4′), 3.95 (s, 2 H, (*E*) H-4′), 4.09–3.40 (m, 2 H), 1.87 and 1.86 (2 s, 3 H, (*E*,*Z*) CH_3), 1.70–1.20 (m, 6 H); MS (D-EI, CH_2Cl_2), *m/e* 303.169 39 (calcd), 303.168 57 (found) (14), 277 (42), 203 (36), 188 (73), 136 (47), 85 (100).

9-[4-[(Benzyloxy)methoxy]-3-[[(benzyloxy)methoxy]methyl]but-2-enyl]adenine (27d). Compound **27d** was prepared in the same manner as **27a**, starting from **25d** (5.0 g, 13.84 mmol). ^{1}H NMR of the intermediate bromide **26d**, ($CDCl_3$) δ 7.43 (s, 10 H), 6.07 (t, 1 H, *J* = 11 Hz), 4.76 (s, 4 H), 4.64 (s, 4 H), 4.30 and 4.23 (2 s, 4 H), 4.10 (d, 2 H, *J* = 11 Hz).

Overall yield of compound 27d: 3.7 g (56%); mp 72–73 °C; ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1 H), 7.78 (s, 1 H), 7.30 (s, 10 H), 5.90 (br s, 2 H, NH_2, exchanged), 5.90 (t, 1 H, *J* = 7 Hz), 4.90 (d, 2 H, *J* = 7 Hz), 4.79 and 4.75 (2 s, 4 H), 4.68 and 4.58 (2 s, 4 H), 4.33 (s, 2 H, (*Z*) H-4′), 4.13 (s, 2 H, (*E*) H-4″); MS (CI, NH_3), *m/e* 476 (100), 384 (35), 203 (11), 136 (37). Anal. ($C_{26}H_{29}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

(*E*,*Z*)-9-[2-[4-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-2-methyl-1,3-dioxanylidene]ethyl]adenine (27e). Compound **27e** was prepared in the same manner as **27a**, starting from compound **25e** (1.0 g, 3.5 mmol) (a mixture of *E* and *Z* isomers). ^{1}H NMR of the intermediate bromide **26e**, ($CDCl_3$) δ 5.85 (t (overlapping), 1 H, (*E*,*Z*) olefin, *J* = 9 Hz), 4.85 (q, 1 H), 4.71 and 4.55 (d, 1 H), 4.17 (d, 2 H, *J* = 9 Hz), 4.11 (d, 1 H, *J* = 6 Hz), 3.82 (dd, 2 H, *J* = 6 Hz), 1.34 (2 d, 3 H, 2 CH_3, isomers), 0.89 (s, 9 H), 0.07 (s, 6 H).

Overall yield of compound 27e: 0.65 g (46%); mp 154–155 °C; $[\alpha]_D$ −144° (*c* 0.95, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1 H), 7.75 (s, 1 H), 6.17 (br s, 2 H, NH_2, exchanged), 5.68 (d, 1 H, *J* = 7 Hz), 4.90 and 4.8 (2 d, 2 H, (*Z*) and (*E*) H-1′, *J* = 7 Hz), 4.35 (s, 2 H, (*Z*) H-6′), 4.22 (s, 2 H, (*E*) H-6′), 4.05 (m, 1 H), 3.83 (dd, 2 H), 1.36 (2 d, 3 H, (*E*,*Z*) CH_3), 0.92 and 0.83 (2 s, 9 H), 0.03 (s, 6 H); MS (D-EI, CH_2Cl_2), *m/e* 405 (14), 304 (53), 188 (40), 135 (35), 85 (100). Anal. ($C_{19}H_{31}N_5O_3Si \cdot {}^1/_8H_2O$) C, H, N.

(*R*)-4-Amino-1-[(*Z*)-3,4-(isopropylidenedioxy)pent-2-enyl]imidazo[4,5-*c*]pyridine (27f). Compound **27f** was prepared in the same manner as **27a**, starting from **25a** (150 mg, 1.0 mmol), except adenine was replaced 3-deazaadenine[27] to yield 0.130 g (47%): mp 122 °C; $[\alpha]_D$ −58.89° (*c* 0.9, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 7.87 (d, 1 H, *J* = 6 Hz), 7.77 (s, 1 H), 6.75 (d, 1 H, *J* = 6 Hz), 5.72 (m, 2 H, *J* = 6 Hz), 5.25 (br s, 2 H, NH_2, exchanged), 4.74 (m, 1 H), 4.17 (dd, 1 H, J_{ab} = 8 Hz), 3.66 (dd, 1 H, J_{ab} = 8 Hz), 1.55 and 1.53 (2 s, 6 H); MS (D-EI, CH_2Cl_2), *m/e* 274 (21), 199 (19), 135 (100). Anal. ($C_{14}H_{18}N_4O_2 \cdot {}^5/_3H_2O$) C, H, N.

4-Amino-1-[4-[(benzyloxy)methoxy]-3-[[(benzyloxy)methoxy]methyl]but-2-enyl]imidazo[4,5-*c*]pyridine (27g). Compound **27g** was prepared in the same manner as **27a**, starting from **25d** (0.36 g, 1.0 mmol), except adenine was replaced with 3-deazaadenine[27] to yield 0.160 g (38%): mp 35 °C, oily solid; ^{1}H NMR ($CDCl_3$) δ 7.84 (dd, 1 H, *J* = 8 Hz), 7.72 (s, 1 H), 7.30 (s, 10 H), 6.71 (d, 1 H, *J* = 8 Hz), 5.81 (t, 1 H, *J* = 7 Hz), 5.30 (br s, 2 H, NH_2, exchanged), 4.88 (s, 4 H), 4.80 (s, 4 H), 4.61 (d, 2 H, *J* = 7 Hz), 4.29 (s, 2 H, (*Z*) H-4′), 4.14 (s, 2 H, (*E*) H-4′); (D-EI, CH_2Cl_2), *m/e* 474.226 52 (calcd), 474.224 97 (found) (14), 353 (20), 223 (40), 199 (70), 134 (25), 91 (100). Anal. ($C_{27}H_{30}N_4O_4$) C, H, N.

9-(3-Methylbut-2-enyl)adenine (6). Compound **6** was made according to the procedure reported by Leonard and Deyrup.[21]

General Procedure for the Deprotection of the Nucleosides. The compound (**27a,b,c,d,e,f,** or **g**) was mixed with 20 mL of H_2O, and 1 mL of 6 N HCl was added. The mixture was stirred at room temperature for 3–6 h until TLC (silica gel plates, CH_2Cl_2/EtOH (9:1)) showed no starting material remained. The solution was concentrated to dryness and the solid was dissolved in 1–2 mL of H_2O and applied to a Dowex 1X8-50(H+) column (except where indicated). The product was eluted with dilute ammonium hydroxide and concentrated to dryness in vacuo. The compounds were dried azeotropically with EtOH.

(*R*)-9-[(*Z*)-3,4-Dihydroxypent-2-enyl]adenine (4a): yield 0.4 g (95%); mp 101–102 °C; $[\alpha]_D$ +35° (*c* 1.0, EtOH); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.16 and 8.12 (2 s, 2 H), 5.74 (m, 2 H, *J* = 13 Hz, *J* = 5 Hz), 4.75 (d, 2 H, *J* = 5 Hz), 3.93 (m, 1 H), 3.34 (d, 2 H, *J* = 5 Hz); MS (D-EI, MeOH), *m/e* 235 (19), 204 (17), 174 (58), 135 (100). Anal. ($C_{10}H_{13}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

(*R*,*S*)-9-[(*E*)-3,4-Dihydroxypent-2-enyl]adenine (5): yield 0.39 g (94%); mp 184–185 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.14 and 8.11 (2 s, 2 H), 5.73 (m, 2 H, *J* = 16 Hz, *J* = 5 Hz), 4.74 (d, 2 H, *J* = 5 Hz), 3.92 (m, 1 H), 3.25 (d, 2 H, *J* = 5 Hz); MS (CI, NH_3), *m/e* 235 (100), 174 (28), 136 (100). Anal. ($C_{10}H_{13}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

(*Z*)-9-(4-Hydroxy-3-methylbut-2-enyl)adenine (7) and (*E*)-9-(4-Hydroxy-3-methylbut-2-enyl)adenine (8): isolated as the hydrochloride salt; yield 0.15 g (89%). The *Z* and *E* isomers were separated with a PRP semipreparative HPLC column with MeOH/H_2O (3:2) as the solvent system. This gave 70 mg of the *Z* compound **7** and 35 mg of the *E* compound **8** (63%, separated yield).

***Z* isomer 7**: mp 170 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.45 and 8.35 (2 s, 2 H), 5.39 (t, 1 H, *J* = 8 Hz), 4.81 (d, 2 H, *J* = 8 Hz), 4.13 (s, 2 H), 1.73 (s, 3 H); MS (D-EI, MeOH), *m/e* 219 (7), 188 (100), 148 (10), 136 (75). Anal. ($C_{10}H_{13}N_5O \cdot HCl \cdot H_2O$) C, H, N.

***E* isomer 8**: mp 178 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.40 (s, 2 H), 5.61 (t, 1 H, *J* = 8 Hz), 4.84 (d, 2 H, *J* = 8 Hz), 3.79 (s, 2 H), 1.73 (s, 3 H); MS (D-EI, MeOH), *m/e* 219.111 92 (calcd), 219.111 78 (found) (24), 188 (100), 135 (54).

9-[4-Hydroxy-3-(hydroxymethyl)but-2-enyl]adenine (9a). The hydrolysis of **27d** required refluxing conditions and the product **9a** was isolated as the hydrochloride salt: yield 0.45 g (88%); mp 176–178 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.45 (s, 2 H), 5.63 (t, 1 H, *J* = 8 Hz), 4.88 (d, 2 H, *J* = 8 Hz), 4.12 (s, 2 H, (*Z*) H-4′), 3.93 (s, 2 H, (*E*) H-4′); MS (CI, NH_3), *m/e* 236 (100), 204 (21), 133 (46). Anal. ($C_{10}H_{13}N_5O_2 \cdot HCl \cdot {}^3/_2H_2O$) C, H, N.

(*E*,*Z*)-9-[4,5-Dihydroxy-3-(hydroxymethyl)pent-2-enyl]adenine (10 and 11): yield 0.10 g (94%); mp 136–137 °C; $[\alpha]_D$ −26.78° (*c* 1.05, MeOH); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.16 (s, 2 H), 5.70 (t (overlapping), 1 H, *J* = 8 Hz), 4.91 (d, 2 H, *J* = 8 Hz), 4.18 (s, 2 H), 3.98 (d, 1 H, *J* = 7 Hz), 3.37 (dd, 2 H, *J* = 7 Hz); MS (D-EI, MeOH), *m/e* 266 (11), 234 (22), 204 (55), 136 (100). Anal. ($C_{11}H_{15}N_5O_3 \cdot EtOH$) C, H, N.

(*R*)-4-Amino-1-[(*Z*)-3,4-dihydroxypent-2-enyl]imidazo[4,5-*c*]pyridine (4b): yield 50 mg (95%); mp 100–101 °C; $[\alpha]_D$ −42.78° (*c* 1.2, MeOH); ^{1}H NMR (CD_3OD) δ 8.16 (s, 1 H), 7.68

(d, 1 H, J = 7 Hz), 6.92 (d, 1 H, J = 7 Hz), 5.71 (m, 2 H, J = 6 Hz), 4.99 (d, 2 H, J = 6 Hz), 4.60 (m, 1 H), 4.55 (d, 2 H, J = 6 Hz); MS (D-EI, MeOH), m/e 234 (26), 203 (10), 173 (10), 148 (13), 134 (100). Anal. ($C_{11}H_{14}N_4O_2 \cdot {}^1/_4H_2O$) C, H, N.

4-Amino-1-[4-hydroxy-3-(hydroxymethyl)but-2-enyl]-imidazo[4,5-*c*]pyridine (9b). Hydrolysis of compound **27g** required refluxing conditions and the product **9b** was isolated as the hydrochloride salt: yield 80 mg (98%), mp 226-227 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.40 (s, 1 H), 7.67 (d, 1 H, J = 8 Hz), 7.22 (d, 1 H, J = 8 Hz), 5.50 (t, 1 H, J = 7 Hz), 5.02 (d, 2 H, J = 7 Hz), 4.13 (s, 2 H, (*Z*) H-4'), 3.95 (s, 2 H, (*E*) H-4'); MS (D-EI, MeOH) m/e 234.111 58 (calcd), 234.112 72 (found) (30), 185 (10), 148 (13), 134 (100).

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Registry No. 1a, 114978-79-9; **1b**, 114979-16-7; **2a**, 114978-80-2; **2b**, 114978-82-4; **3a**, 104715-57-3; **3b**, 114978-83-5; **4a**, 114979-05-4; **4b**, 114979-13-4; **5**, 115016-40-5; **6**, 5122-38-3; **7**, 114979-06-5; **7**·HCl, 114979-08-7; **8**, 114979-07-6; **8**·HCl, 114979-09-8; **9a**, 114979-10-1; **9a**·HCl, 107053-43-0; **9b**, 114979-14-5; **9b**·HCl, 114979-15-6; **10**, 114979-11-2; **11**, 114979-12-3; **12a**, 114978-78-8; **12b**, 114978-81-3; **13**, 114978-77-7; **14**, 22323-80-4; **15a**, 106757-56-6; **15b**, 85881-88-5; (*E*)-**15c**, 74094-41-0; (*Z*)-**15c**, 74094-42-1; **15d**, 114978-87-9; **15e**, 114978-88-0; **16**, 22323-83-7; **17**, 66183-63-9; **18**, 116-09-6; **19**, 53343-13-8; **20**, 96-26-4; **21**, 114978-84-6; **22**, 6207-26-7; **23**, 114978-85-7; **24**, 114978-86-8; **25a**, 106757-58-8; **25b**, 115016-37-0; (*E*)-**25c**, 114978-89-1; (*Z*)-**25c**, 114978-90-4; **25d**, 114978-91-5; **25e**, 114978-92-6; **26a**, 114978-93-7; **26b**, 115016-38-1; (*E*)-**26c**, 114978-95-9; (*Z*)-**26c**, 114978-96-0; **26d**, 114978-99-3; **26e**, 114979-01-0; **27a**, 114978-94-8; **27b**, 115016-39-2; (*E*)-**27c**, 114978-97-1; (*Z*)-**27c**, 114978-98-2; **27d**, 114979-00-9; **27e**, 114979-02-1; **27f**, 114979-03-2; **27g**, 114979-04-3; $Ph_3P{=}CHCO_2Me$, 2605-67-6; $(MeO)_2POCH_2CO_2Me$, 5927-18-4; EC 3.3.1.1, 9025-54-1; adenine, 73-24-5; 3-deazaadenine, 6811-77-4; sodium adenine, 52994-56-6.

Thienylpyrazoloquinolines: Potent Agonists and Inverse Agonists to Benzodiazepine Receptors

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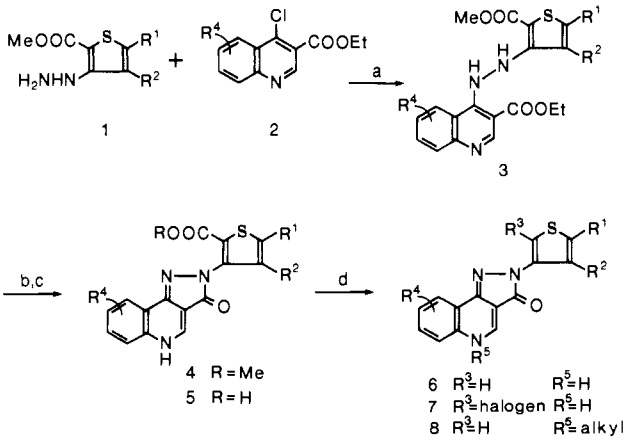
Synthesis and structure-activity relationships of a series of 2-(thien-3-yl)- and 2-(thien-2-yl)-2,5-dihydro-3*H*-pyrazolo[4,3-c]quinolin-3-ones are reported. A number of the compounds possessed 1 order of magnitude higher affinity for the receptors than diazepam. Planarity was one of the structural requirements for binding to benzodiazepine receptors. The activities of agonists and inverse agonists were assessed on the basis of inhibition or facilitation of the pentylenetetrazole-induced convulsions, respectively. Thien-3-yl compounds exhibited inverse agonist activity whereas thien-2-yl analogues with a 5'-alkyl group showed agonist activity. Substitution on the quinoline moiety did not enhance in vivo activity. The most potent compounds were the 5-methylthien-3-yl derivative **6a** as an inverse agonist and the 5-methylthien-2-yl compound **13a** as an agonist.

Since the discovery of benzodiazepine (BZ) receptors in the central nervous system,[1] rapid advances have been made toward understanding the mechanism of benzodiazepine action.[2] The BZ receptor as an allosteric regulatory site of the γ-aminobutyric acid (GABA) receptor is considered to mediate two opposing effects, one to amplify or facilitate the action of GABA ("positive" efficacy[3]), the other to reduce the action of GABA ("negative" efficacy); by convention, agents facilitating GABA-receptor/Cl^- channel function via the BZ receptor are called agonists, and agents reducing the receptor/channel function have been termed inverse agonists.[3,4] Antagonists at BZ receptors block the effect of both agonists and inverse agonists by competitively inhibiting their binding to the BZ receptor. Diazepam, β-CCM,[5] and Ro 15-1788[6] are representative of an agonist, an inverse agonist, and an antagonist, respectively. Generally, the agonists have anxiolytic and anticonvulsant properties, while the inverse agonists exhibit anxiogenic and convulsant effects. From detailed pharmacological investigations, Braestrup et al.[7a] have suggested that BZ receptor ligands in fact comprise a continuous spectrum of agents with a graduated variety of efficacy at the receptor: (1) full agonists, (2) partial agonists, (3) pure agonists, (4) partial inverse agonists, and (5) full inverse agonists. They also demonstrated that some β-carboline derivatives exhibit a wide spectrum by changing the substituents on the parent skeleton. Yokoyama et al.[8] have reported that the phenylpyrazolo-

Scheme I[a]



[a] (a) EtOH/room temperature. (b) 1 N aqueous NaOH/EtOH/room temperature. (c) 1 N aqueous NaOH/EtOH/reflux. (d) Cu/quinoline/190 °C.

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