Adenosine Analogues as Selective Inhibitors of Glyceraldehyde-3-phosphate Dehydrogenase of *Trypanosomatidae* via Structure-Based Drug Design

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In our continuation of the structure-based design of anti-trypanosomatid drugs, parasite-selective adenosine analogues were identified as low micromolar inhibitors of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Crystal structures of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania mexicana*, and human GAPDH's provided details of how the adenosyl moiety of NAD^+ interacts with the proteins, and this facilitated the understanding of the relative affinities of a series of adenosine analogues for the various GAPDH's. From exploration of modifications of the naphthalenemethyl and benzamide substituents of a lead compound, N^6-(1-naphthalenemethyl)-2′-deoxy-2′-(3-methoxybenzamido)adenosine (**6e**), N^6-(substituted-naphthalenemethyl)-2′-deoxy-2′-(substituted-benzamido)adenosine analogues were investigated. N^6-(1-Naphthalenemethyl)-2′-deoxy-2′-(3,5-dimethoxybenzamido)adenosine (**6m**), N^6-[1-(3-hydroxynaphthalene)methyl]-2′-deoxy-2′-(3,5-dimethoxybenzamido)adenosine (**7m**), N^6-[1-(3-methoxynaphthalene)methyl]-2′-deoxy-2′-(3,5-dimethoxybenzamido)adenosine (**9m**), N^6-(2-naphthalenemethyl)-2′-deoxy-2′-(3-methoxybenzamido)adenosine (**11e**), and N^6-(2-naphthalenemethyl)-2′-deoxy-2′-(3,5-dimethoxybenzamido)adenosine (**11m**) demonstrated a 2- to 3-fold improvement over **6e** and a 7100- to 25000-fold improvement over the adenosine template. IC_{50}'s of these compounds were in the range 2−12 μM for *T. brucei*, *T. cruzi*, and *L. mexicana* GAPDH's, and these compounds did not inhibit mammalian GAPDH when tested at their solubility limit. To explore more thoroughly the structure−activity relationships of this class of compounds, a library of 240 N^6-(substituted)-2′-deoxy-2′-(amido)adenosine analogues was generated using parallel solution-phase synthesis with N^6 and C2′ substituents chosen on the basis of computational docking scores. This resulted in the identification of 40 additional compounds that inhibit parasite GAPDH's in the low micromolar range. We also explored adenosine analogues containing 5′-amido substituents and found that 2′,5′-dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(diphenylacetamido)adenosine (**49**) displays an IC_{50} of 60−100 μM against the three parasite GAPDH's.

Introduction

According to the World Health Organization, *Trypanosoma brucei*, the causative agent of African sleeping sickness, infected 300 000 to 500 000 people in 1999 and poses an escalating health threat to nearly 60 million people of 36 countries of sub-Saharan Africa.[1,2] Introduced into the mammalian host via the blood-meal bite of the tsetse fly vector, *T. brucei* freely replicates in the bloodstream as it makes its way to the central nervous system (CNS). From there, this parasitic infection induces delirium, seizures, coma, and, if left untreated, death.[3] Existing drug therapies, including suramin, pentamidine, melarsoprol, and difluoromethylornithine (DFMO), are inadequate because they suffer from one or more drawbacks such as ineffectiveness against more virulent strains, ineffectiveness against post-CNS invasion, toxicity, resistance, or parenteral administration.[4,5] Other diseases caused by *Trypanosomatidae*, which share a similar state with respect to available drug treatment, include Chagas' disease (*Trypanosoma cruzi*)[6] and leishmaniasis (*Leishmania spp.*).[7] Clearly, there exists a need for safer and more efficacious chemotherapeutic agents against trypanosomatids.

The focus of this work is on glycolysis in trypanosomes as a target for structure-based drug design. Rigorous studies of energy metabolism in *T. brucei* have established that, unlike the insect form, the bloodstream form lacks a functional TCA cycle and mitochondrial oxidative phosphorylation and depends solely on glycolysis with the excretion of pyruvate for energy production.[8−10] As has been shown by Clarkson and Brohn, treatment of cultured parasites with combinations of glycerol and salicylhydroxamic acid causes inhibition of carbohydrate catabolism and severely impedes parasite proliferation.[11] Computer modeling of *T. brucei*'s glycolytic flux suggests that, unlike in mammalian cells, glycosomal

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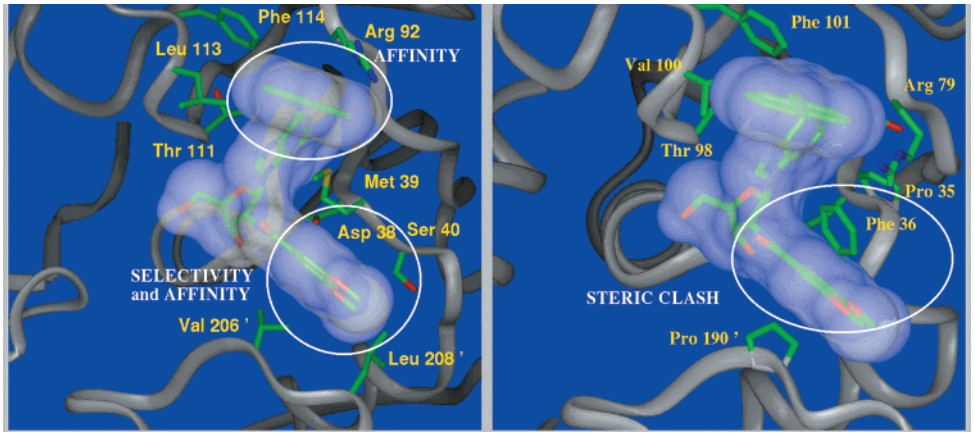


Figure 1. (Left) *L. mexicana* GAPDH/**6m** crystal structure. Circles denote the cleft recognized by the 2′-substituent, which provides for selectivity and affinity, while the N^6 region provides for affinity. In human GAPDH (right), recognition of the C2′ substituent is precluded by steric occlusion.

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exerts significant control over the glycolytic pathway. Competitive inhibitors of this enzyme, when present at concentrations 10- to 100-fold higher than their K_I values, are predicted to reduce parasite glycolytic flux to zero.[12−14] Energy metabolism in *Leishmania spp.* and in *T. cruzi* has been studied predominantly with the promastigote and epimastigote forms, respectively, because the intracellular forms are difficult to analyze because of interference from host cell metabolism. However, glycolysis is always active in these parasites and biochemical studies with the *T. cruzi* axenic amastigote intracellular form suggest that carbohydrate catabolism is its major source of energy.[15] Thus, glycolysis inhibitors have the potential to serve as effective anti-*T. cruzi* and anti-*Leishmania* agents.

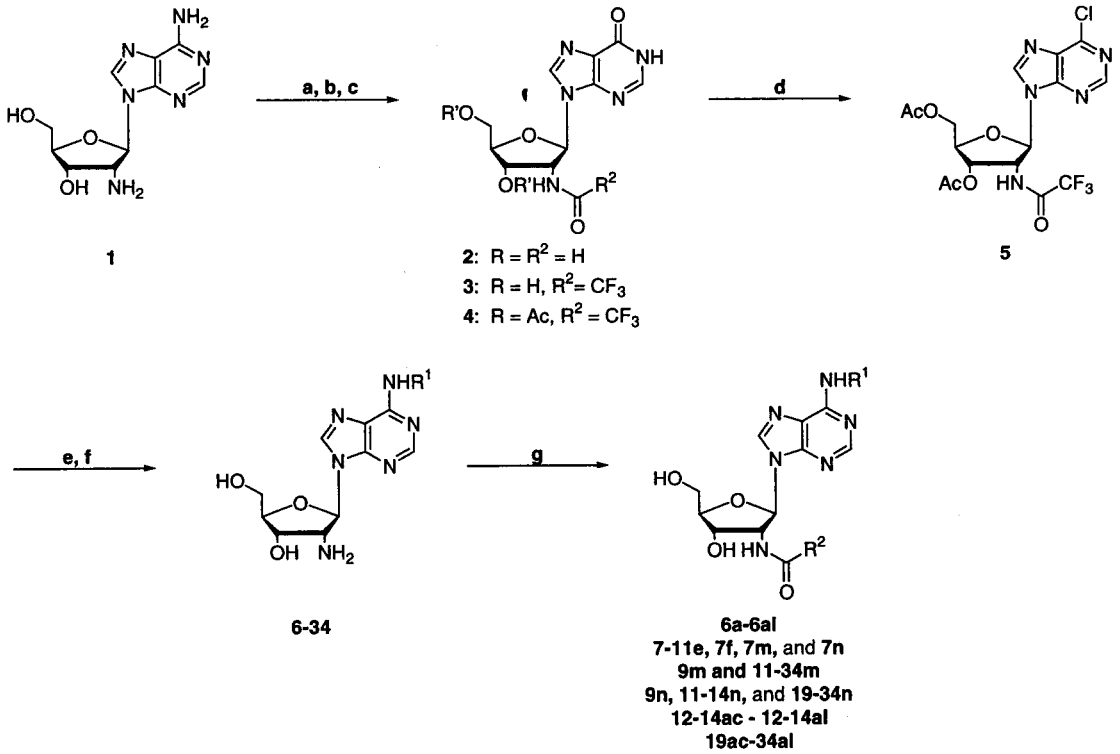
Guided by crystal structures of *T. brucei*, *T. cruzi*, and *L. mexicana* GAPDH's[16−18] in comparison with human GAPDH,[16,19] the process of designing competitive and selective inhibitors is facilitated. X-ray structures co-bound with their natural substrates reveal the detailed binding mode of the adenosyl moiety of the NAD^+ cosubstrate and have led to the successful design of adenosine analogues as selective and competitive inhibitors of trypanosomatid GAPDH's.[20−24] Although the active sites of the parasite and mammalian enzymes are highly conserved in the region that binds glyceraldehyde-3-phosphate, the adenosyl moiety is well removed from the catalytic cysteine and sits in a region that is far less conserved between parasite and mammalian enzymes. A detailed description of the targeted region has been described previously.[21] The adenosine scaffold provides several opportunities not only for improving affinity but also for improving selectivity. Specifically, a hydrophobic cleft protruding from the ribosyl C2′ is a hallmark of the trypanosomatid GAPDH's. This "selectivity cleft" is absent from the human enzyme because of a difference in protein backbone conformation. The area around the adenosyl N^6 exhibits an additional surface for hydrophobic substituents in all enzymes, though the region appears somewhat larger in the trypanosomatid GAPDH's. Both mentioned opportunities were exploited previously by our group, culminating in the design of a trypanosomatid-selective inhibitor N^6-(1-naphthalenemethyl)-2′-deoxy-2′-(3-methoxybenzamido)adenosine (**6e**)[24] with IC_{50} values that have been determined to be 25, 12, and 6 μM for *T. brucei*, *T. cruzi*, and *L. mexicana* GAPDH's, respectively.

In this paper, the exploration of the C2′ and N^6 regions is continued with a structure−activity relationship (SAR) series of benzamides and with modifications to the naphthalene ring, respectively. To further investigate the diversity of substituents that could be accommodated by these two regions, a 2.8 Å X-ray structure of an *L. mexicana* GAPDH/adenosine analogue complex (Figure 1)[25] was used to generate a combinatorial library of 240 N^6-(substituted)-2′-deoxy-2′-(amido)adenosine derivatives, via computational docking methodology. Additionally, the incorporation of 5′-amido substituents into adenosine analogues bearing either of the most potent N^6 or C2′ substituents is explored.

Chemistry

There are three reported methods for the synthesis of N^6-(substituted)-2′-deoxy-2′-(benzamido)adenosine analogues: (1) acylation of 2′-amino-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine[20,26] followed by N1 alkylation with subsequent Dimroth rearrangement and removal of the Markiewicz protecting group;[23,27] (2) acylation of 2′-amino-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine followed by diazotization−iodination of N^6, C6 amination, and removal of the Markiewicz protecting group;[24] (3) diazotization−chlorination of 2′-azido-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine, removal of the Markiewicz protecting group, amination of the C6 position, reduction of the C2′ azide, and polymer-assisted amidation.[28] Route 1 suffers from poor yields of the alkylation and rearrangement steps and is limited in scope to the availability, either commercially or synthetically, of activated halide alkylating agents. Routes 2 and 3 both suffer from poor yields at the diazotization−halogenation step. The acylation step of route 3 is attractive because the desired end-product can simply be washed cleanly from the resin; however, the resin adds two additional steps to the synthesis because it also needs to be prepared.[29,30] To more thoroughly explore N^6,2′-disubstituted adenosine analogues, we needed to develop an economically robust route to a difunctionalized intermediate that would allow for the incorporation of a wide variety of substituents at both the N^6 and C2′ positions.

Scheme 1[a]



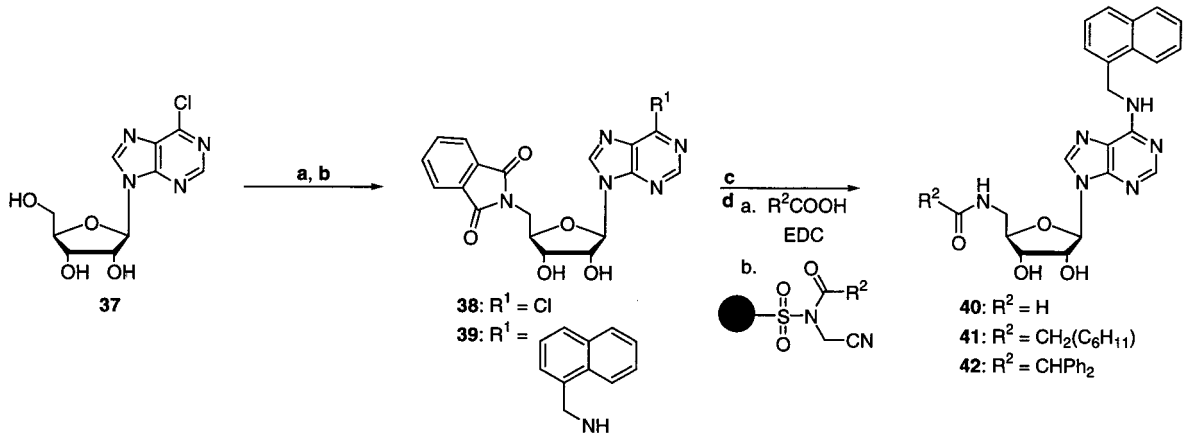
[a] (a) Adenosine deaminase in H_2O at room temperature; (b) $EtOC(O)CF_3$ in DMF at 60 °C; (c) Ac_2O, Et_3N, and DMAP in ACN at room temperature; (d) *N*,*N*-dimethylaniline, $POCl_3$, and Et_4NCl in ACN; (e) R^1NH_2 and Et_3N in EtOH at 60 °C; (f) NH_4OH in EtOH at 60 °C; (g) R^2COOH and EDCI in CH_2Cl_2/DMF at room temperature.

Our route (Scheme 1) starts with the synthesis of 2′-deoxy-2′-aminoadenosine (**1**)[20,26] and its quantitative conversion to 2′-deoxy-2′-aminoinosine (**2**) by preparative use of adenosine deaminase.[31] Trifluoroacetylation of the C2′ amine and acetylation of the C3′ and C5′ hydroxyls to yield 2′-deoxy-2′-trifluoroacetamidoinosine (**3**) and 2′-deoxy-2′-trifluoroacetamido-3′,5′-O-(diacetyl)-inosine (**4**), respectively, is accomplished in greater than 95% yield for each step. By use of the methodology of Matsuda et al.,[32] subsequent chlorination of the C6 position of **4** with phosphorus oxychloride to yield 9-[2′-deoxy-2′-trifluoroacetamido-3′,5′-O-(diacetyl)]-(1-β-D-ribofuranosyl)-6-chloropurine (**5**) is accomplished in better than 90% yield. Starting from the commercially available arabinose adenosine and transforming through nine steps, the intermediate **5** can be synthesized in 65% overall yield. Amination of the C6 position, in situ removal of the acetates, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDCI) mediated acylation of the C2′ amino yield the desired disubstituted products (Scheme 1; **6a**−**6al**, **7e**−**11e**, **7f**, **7m**, **9m**, **11m**−**34m**, **7n**, **9n**, **11**−**14n**, **19**−**34n**, **12**−**14ac** to **12**−**14al**, and **19ac**−**34al**). Typically, amination of **5** led to a variety of deacetylated products as determined by electrospray ionization mass spectrometry (ESI-MS), but this was of no adverse consequense.

Synthesis of N^6-(1-naphthalenemethyl)-5′-deoxy-5′-(amido)adenosine analogues (Scheme 2) starts with the conversion of commercially available 6-chloropurine-9-riboside (**37**) to 6-chloropurine-9-[5′-deoxy-5′-(phthalimido)]riboside (**38**) via standard Mitsunobu conditions. Amination at C6 followed by in situ hydrazine-mediated cleavage of the phthalimide group yields the respective N^6-(1-naphthalenemethyl)-5′-deoxy-5′-(phthalimido)adenosine (**39**) and N^6-(1-naphthalenemethyl)-5′-deoxy-5′-aminoadenosine (**40**) intermediates. EDCI-mediated acylation or polymer-assisted acylation with the appropriately acylated safety-catch resin[29,30] is chemoselective and gives the desired products N^6-(1-naphthalenemethyl)-5′-deoxy-5′-(cyclohexylacetamido)adenosine (**41**) and N^6-(1-naphthalenemethyl)-5′-deoxy-5′-(diphenylacetamido)adenosine (**42**) in high yield (Scheme 2). Despite the additional steps needed to prepare the resin, employing the safety-catch acylating agent has the added benefit of merely filtering off the solid support to obtain the desired product in nearly quantitative yield and in greater than 95% purity as determined by HPLC.

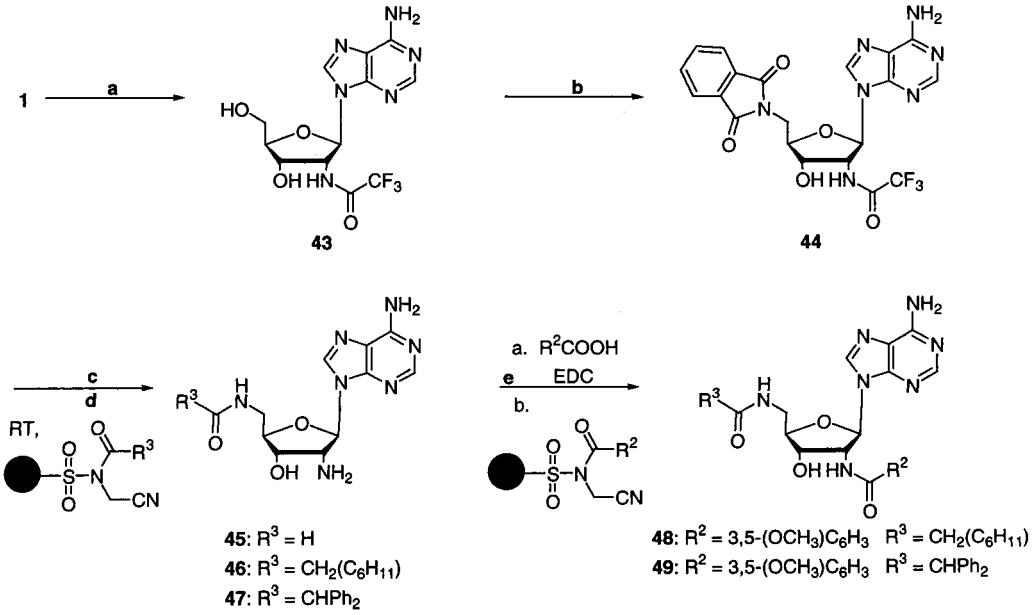
Synthesis of 2′,5′-dideoxy-2′,5′-(bisamido)adenosine analogues also starts with **1** (Scheme 3). Trifluoroacetylation of the 2′ amine yields 2′-deoxy-2′-(trifluoroacetamido)adenosine (**43**). From use of standard Mitsunobu conditions, **43** is converted to 2′,5′-dideoxy-2′-(trifluoroacetamido)-5′-(phthalimido)adenosine (**44**), and subsequent in situ hydrazine-mediated cleavage of both the phthalimide and the trifluoroacetamide protecting group yields the desired 2′,5′-dideoxy-2′,5′-diaminoadenosine intermediate (**45**). Regioselective acylation of the 5′ amine is accomplished with the use of the appropriately acylated safety-catch resin at ambient temperature to give 2′,5′-dideoxy-2′-amino-5′-(cyclohexylacetamido)adenosine (**46**) and 2′,5′-dideoxy-2′-amino-5′-(diphenylacetamido)adenosine (**47**) in greater than 95% yield. EDCI-mediated acylation of the 2′ amine gives the final products 2′,5′-dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(cyclohexylacetamido)adenosine (**48**) and 2′,5′-dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(diphenylacetamido)adenosine (**49**) (Scheme 3). Alternatively, acylation of **46** and **47** can be accomplished by employ-

Scheme 2[a]



[a] (a) Phthalimide, Ph_3P, and DIAD in THF at room temperature; (b) R^1NH_2 and Et_3N in EtOH at 60 °C; (c) $NH_2NH_2 \cdot H_2O$ in EtOH at 80 °C; (d) method a, R^2COOH and EDCI in CH_2Cl_2/DMF at room temperature, or method b, appropriately acylated safety-catch resin in DMF at room temperature.

Scheme 3[a]



[a] (a) $EtOC(O)CF_3$ in DMF at 60 °C; (b) phthalimide, Ph_3P, and DIAD in THF at room temperature; (c) $NH_2NH_2 \cdot H_2O$ in EtOH at 80 °C; (d) appropriately acylated safety-catch resin in DMF at room temperature; (e) method a, R^2COOH and EDCI in CH_2Cl_2/DMF at room temperature, or method b, appropriately acylated safety-catch resin in DMF at 60 °C.

ing the appropriately acetylated safety-catch resin at elevated temperatures.[28,33,34]

2-Naphthalenemethylamine hydrochloride (**51**), 4-methoxy-1-naphthalenemethylamine hydrochloride (**55**), and 3-methoxy-1-naphthalenemethylamine hydrochloride (**57**) were prepared by $LiAlH_4$ reduction of 2-naphthonitrile (**50**),[35] 4-methoxy-1-naphthonitrile (**54**), and 3-methoxy-1-naphthonitrile (**56**),[36] respectively. By use of the methodology of Deana et al.[37,38] for the synthesis of 2-hydroxy-1-naphthalenemethylamine, 2-methoxy-1-naphthalenemethylamine (**53**) was prepared by treatment of 2-methoxynaphthalene (**52**) with 2-chloro-*N*-(hydroxymethyl)acetamide and hydrochloric acid. 3-Hydroxy-1-naphthalenemethylamine hydrobromide (**58**) was prepared by treatment of **57** with BBr_3 (Scheme 4).

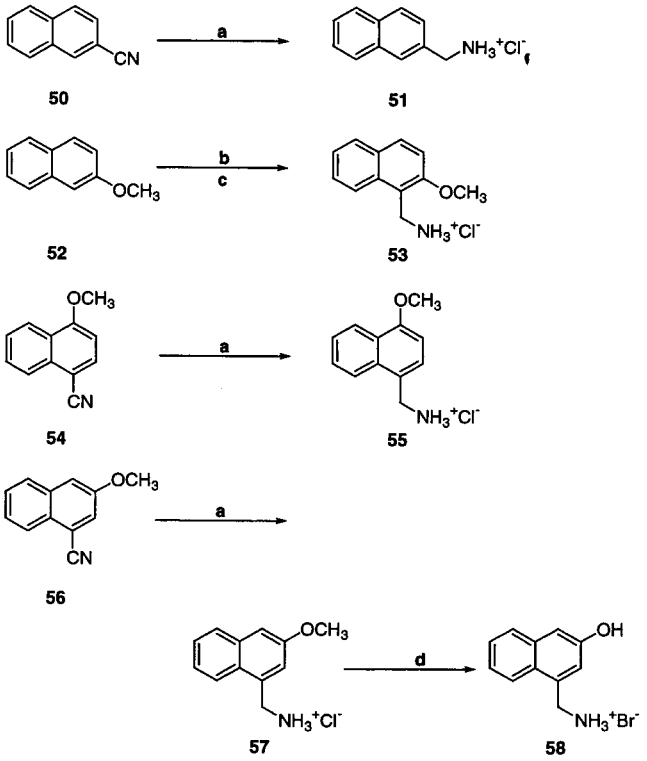
Hydroxylated benzoic acids needed for the synthesis of compounds **6c**, **6g**, **6k**, **6o**, **6r**, **6t**, **6v**, and **6x** were acetylated under standard conditions.[39] After they were coupled to the nucleoside, deprotection of the phenolic groups was accomplished in methanolic ammonia.

Results and Discussion

All inhibitors were screened against *L. mexicana* GAPDH (Tables 1−5). Additionally, all of the compounds were docked into the structure of *L. mexicana* GAPDH, which was derived from the X-ray structure of the complex with N^6-(1-naphthalenemethyl)-2′-deoxy-2′-(3,5-dimethoxybenzamido)adenosine (**6m**) after removal of the inhibitor coordinates.[25] Docking studies were performed with the program FLO in which molecular potential energy calculations with the QXP force field are accessible through a graphical interface.[40] With this information, we attempted to rationalize the observed inhibition data.

Initially we docked the inhibitor of the crystallographic complex, compound **6m**, to verify whether the program could reproduce the experimentally observed

Scheme 4[a]



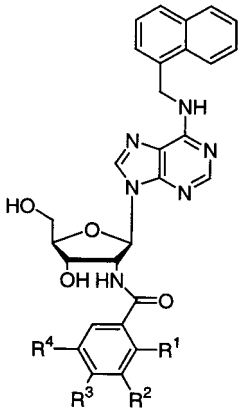
[a] (a) $LiAlH_4$ in THF at 0 °C; (b) $ClCH_2CONHCH_2OH$ and H^+ in EtOH at 80 °C; (c) [HCl] in EtOH at 80 °C; (d) BBr_3 in CH_2Cl_2 at 40 °C.

binding mode. After docking, the root-mean-square (rms) deviation between the experimental coordinates and the docked model was only 0.70 Å for 42 atoms, confirming the validity of the docking protocol. This rms deviation is similar to the value of 0.76 Å found by the FLO authors for a diverse set of unrelated test cases. Subsequently, all other inhibitors were docked using the same protocol. The calculated binding energies (data not shown) comprise ligand and protein strain energies, van der Waals energy, electrostatic interaction energy (distance-dependent dielectric is $4R\epsilon$), and a term accounting for hydrophobic interaction. Because there is no explicit penalty term for desolvation of hydrophilic groups, the calculated interaction energies allow only for qualitative interpretation of ligand binding,[41] as clearly demonstrated in a design study of potent angiotensin-converting enzyme (ACE) inhibitors.

Modifications of the C2′ Benzamide. To more thoroughly explore the conformational space of the C2′ selectivity cleft, an SAR of N^6-(1-naphthalenemethyl)-2′-deoxy-2′-(benzamido)adenosine analogues was generated. This series of compounds incorporates methoxy, chloro, acetyl, and hydroxy groups at various positions on the benzamide ring.

Of the N^6-naphthalenemethyl-2′-benzamido analogues, the most drastic decreases in binding affinity were noted with 2-substituted benzamides that had IC_{50}'s greater than 100 μM (Table 1). In principle, two orientations of the benzamide fragments are possible: syn, which places the ortho substituent adjacent to the amide carbonyl oxygen and pointing into solvent; and anti, which places the ortho substituent opposite the carbonyl oxygen pointing into the selectivity cleft. Modeling experiments suggest the syn conformation is

Table 1. Inhibition of *L. mexicana* GAPDH by N^6-(1-Naphthalenemethyl)-2′-deoxy-2′-(benzamido)adenosine Analogues[a]



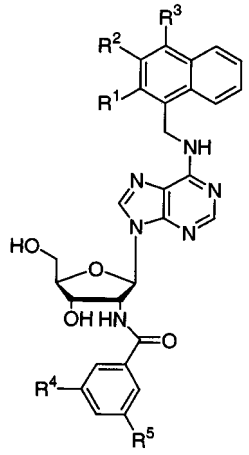
6a-6z

compd	R^1	R^2	R^3	R^4	IC_{50} (μM)
6a	OCH_3	H	H	H	inactive[b]
6b	Cl	H	H	H	inactive
6c	OAc	H	H	H	inactive
6d	OH	H	H	H	inactive
6e	H	OCH_3	H	H	6
6f	H	Cl	H	H	6
6g	H	OAc	H	H	50
6h	H	OH	H	H	25
6i	H	H	OCH_3	H	100
6j	H	H	Cl	H	100
6k	H	H	OAc	H	100
6l	H	H	OH	H	80
6m	H	OCH_3	H	OCH_3	2
6n	H	Cl	H	Cl	12
6o	H	OAc	H	OAc	10
6p	H	OH	H	OH	5
6q	H	OCH_3	OCH_3	H	4
6r	H	OAc	OAc	H	25
6s	H	OH	OH	H	18
6t	OAc	OCH_3	H	H	inactive
6u	OH	OCH_3	H	H	inactive
6v	H	OAc	OCH_3	H	78
6w	H	OH	OCH_3	H	37
6x	H	OCH_3	OAc	H	10
6y	H	OCH_3	OH	H	10
6z	H	$N(CH_3)_2$	H	H	25

[a] For compounds with IC_{50}'s $\leq$ 50 μM, inhibitor concentrations were varied, with at least five different concentrations used to determine the IC_{50} values. Statistical error limits on the IC_{50} values have been calculated and amount to 10% or less. For compounds with IC_{50}'s > 50 μM, these values were extrapolated from at least three different concentrations. [b] Inactive = inactive at 50 μM.

unlikely because the lone pairs of the two oxygen atoms point unfavorably toward each other. Work by Cignitti et al. would support this observation,[42] and a Cambridge Structural Database (CSD)[43] search for *o*-methoxy- and *o*-chlorobenzamides reveals that the 26 available crystal structures all exhibit the anti conformation. In contrast, the anti conformation allows the methoxy and hydroxy substituents to make a 2.7 Å hydrogen bond with the amide nitrogen. Docking of the anti conformation of the ortho-substituted compounds by FLO reveals that the benzamido is pushed out of the selectivity cleft by 1.3−1.8 Å compared to that of **6m** because of steric constraints. The end result is that a key hydrogen bond between the amide nitrogen and the carboxylate of Asp-38 is significantly weakened, and the benzamide ring buries less hydrophobic surface in the cleft. This most likely explains the poor activity of these compounds.

Table 2. Inhibition of *L. mexicana* GAPDH by N^6-Naphthalenemethyl-2′-deoxy-2′-(3-methoxybenzamido)-adenosine Analogues



7e-10e
7f, 7m, and 7n
9m and 9n

compd	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)
7e	H	OH	H	OCH_3	H	6
7f	H	OH	H	Cl	H	4
7m	H	OH	H	OCH_3	OCH_3	2
7n	H	OH	H	Cl	Cl	25
8e	OCH_3	H	H	OCH_3	H	inactive[a]
9e	H	OCH_3	H	OCH_3	H	3
9m	H	OCH_3	H	OCH_3	OCH_3	2
9n	H	OCH_3	H	Cl	Cl	25
10e	H	H	OCH_3	OCH_3	H	6

[a] Inactive = inactive at 50 μM.

With the exceptions of **6e** and **6f**, for which affinities had been previously determined,[24] the meta- and para-substituted benzamides (Table 1) displayed IC_{50}'s of 25−100 μM against *L. mexicana* GAPDH. Essentially, these compounds all had the same coordinates as **6m** and FLO preferentially oriented the meta substituents to point into the selectivity cleft. Compounds **6m**, **6o**, and **6p**, with a 3,5-disubstituted benzamido substituent, exhibited a 3- to 5-fold increase in affinity for *L. mexicana* GAPDH when compared to their 3-substituted analogues **6e**, **6g**, and **6h**, while **6n** exhibited a 2-fold decrease in activity. The marginal differences in the inhibitor potency suggests that the 3-monosubstituted benzamides can adopt either the anti or syn conformation. Compound **6z** was synthesized in an attempt to more completely fill the "selectivity cleft" and to explore alternative conformations of the methoxy group of the lead inhibitor **6e**. Modeling suggested that replacing methoxy with dimethylamino might accomplish that goal, but the IC_{50} of **6z** was 4 times worse than **6e**.

Modifications of the N^6-Naphthalenemethyl. Computational modeling experiments suggested that a hydroxyl group incorporated into the 3-position of the naphthyl ring or a methoxy group in the 3- or 4-position of the naphthyl ring might be well tolerated or might enhance binding affinity of the lead compound **6e** (Table 2). The hydrogen bond provided by N^6-(3-hydroxy-1-naphthalenemethyl)-2′-deoxy-2′-(3-methoxybenzamido)-adenosine (**7e**) to the backbone carbonyl of Ala-90 was well tolerated, and the compound displayed an IC_{50} of 6 μM. The 3-hydroxynaphthalenemethyl substituent was also incorporated into compounds bearing other C2′ benzamides that were well tolerated by *L. mexicana* GAPDH (3-chlorobenzamide, 3,5-dimethoxybenzamide, and 3,5-dichlorobenzamide), but there were only marginal improvements or, in the case of **7n**, a marginal decrease in affinities. Comparable results were also obtained with the incorporation of the methoxy group into the 3- and 4-positions of the naphthalene ring, and docking experiments with FLO place all of these compounds such that they essentially superimpose with **6m**. Integration of the methoxy group into the 2-position of the naphthyl ring, yielding **8e**, was predicted to drastically decrease binding affinity because the methoxy would clash with either the adenosyl N^6 or the residues Ala-90 and Gln-91 of the protein backbone. In fact, FLO displaced the entire molecule partially out of the binding site. The 2-methoxynaphthalenemethyl analogue (**8e**) was synthesized as proof of principle, and it was inactive against *L. mexicana* GAPDH when tested up to its solubility limit of 50 μM.

An alternative connectivity of the naphthalene ring was explored with the incorporation of 2-naphthalenemethyl and 2-naphthalene substituents. Compounds **11e**, **11m**, and **11n** were well tolerated, especially **11e** and **11m** with IC_{50}'s of 2 μM. FLO docked these compounds with their benzamido adenosine fragments in the same position as in **6m**, and the analysis showed that the β-naphthyl ring makes favorable contacts with the guanadinium of Trp-92. Docking of **18m** (IC_{50} = 60 μM), which has a β-naphthyl directly attached to N^6, elucidated poor packing of the naphthalene ring with the aliphatic portion of Arg-92 and the side chains of Met-39 and Leu-113, which caused a small rotation of the purine ring and a pivoting of the C1′ of the ribose.

Modifications of the N^6 Position. Incorporation of N^6 and C2′ amido substituents into one adenosine scaffold reveals that there is a degree of "cross-talk" between these two regions. For example, N^6-benzyladenosine and N^6-(1-naphthalenemethyl)adenosine have IC_{50}'s of 4 and 0.15 mM, respectively.[23] Despite the 27-fold difference in binding affinity between these two compounds, incorporation of the C2′-(3-methoxy)benzamide into both of them resulted in compounds showing only a 3-fold affinity difference; the IC_{50} of N^6-benzyl-2′-deoxy-2′-(3-methoxybenzamido)adenosine for *L. mexicana* GAPDH is 16 μM,[23] and the IC_{50} of N^6-(1-naphthalenemethyl)-2′-deoxy-2′-(3-methoxybenzamido)adenosine for *L. mexicana* GAPDH is 6 μM. A variety of N^6-monosubstituted adenosine analogues have been reported that exhibited IC_{50}'s against *L. mexicana* GAPDH in the range 4−0.15 mM (N^6-(2-methylbenzyl)-adenosine, IC_{50} = 0.7 mM; N^6-(3-methylbenzyl)adenosine, IC_{50} = 0.7 mM; N^6-(1,2,3,4-tetrahydro-1-naphthyl)-adenosine, IC_{50} = 0.36 mM; N^6-[(2-(hydroxymethyl)-phenylthio)benzyl]adenosine, IC_{50} = 0.34 mM; N^6-(diphenylmethyl)adenosine, IC_{50} = 0.24 mM).[23] We wished to see if these too would exhibit the same increase in binding affinity as N^6-benzyladenosine and N^6-(1-naphthalenemethyl)adenosine when a well-tolerated C2′ benzamide, 3,5-dimethoxybenzamido, was incorporated into the molecule.

A combination of benzyl (**12m**), 2-methylbenzyl (**13m**), or 3-methylbenzyl (**14m**) with C2′-(3,5-dimethoxybenzamide) resulted in activities in the 20−25 μM range. The diphenylmethyl (**15m**), tetrahydronaphthyl (**16m**),

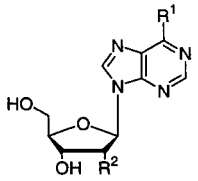
and 2-(2-(hydroxymethyl)phenylthio)benzyl (**17m**) N^6 analogues exhibited IC_{50}'s in the range 55−75 μM. All of these N^6 substituents that exhibited some modest binding affinity toward parasite GAPDH as monosubstituted adenosine analogues did exhibit some degree of "cross-talk" when incorporated into analogues bearing the C2′ benzamide group, yielding tighter binding derivatives, but none better than what had previously been obtained with the incorporation of the naphthalene ring, **6m**.

***N*^{6}-(Substituted)-2′-deoxy-2′-(amido)adenosine Combinatorial Library.** According to the methodology and criteria of Aronov et al.,[44] UC_Select,[45] in conjunction with the Daylight version of the Available Chemicals Directory (ACD), was used to select primary amines and carboxylic acids that were devoid of undesirable pharmaceutical and chemical properties (i.e., substituents without phosphates or sulfates). Sybyl's CONCORD module (version 6.5, 1999; Tripos Associates, St. Louis, MO) was used to generate the three-dimensional coordinates of substituent libraries that were then fused to the adenosyl template. When the coordinates of **6m**, from the *L. mexicana* GAPDH complex, were used, the amine test library (consisting of approximately 460 amines) was fused to the C6 position of **6m** after the removal of the naphthalenemethyl substituent. Likewise, the carboxylic acid test library (consisting of approximately 1400 acids) was fused to the C2′ position of **6m** after the removal of the dimethoxybenzamide. DOCK 4.01[46] was used as previously described[44] to evaluate the potential binding energies of the candidate adenosine derivatives to GAPDH, and then a scoring algorithm that takes into account differential solvation of the adenosine substituents was applied.[47] The best scoring molecules were visually inspected using Sybyl and Insight II (version 98.0, 1998; Molecular Simulations, Inc., San Diego, CA) software packages.

Our initial hope in venturing into the exploration of the virtual library described above was to discover substituents that were structurally different from naphthylenemethyl and benzoyl and could be accommodated in the N^6 and C2′ regions of parasite GAPDH's. This hope was not realized because many of the top DOCK-ranked amines had benzyl and naphthalenemethyl substituents, or substituents that were structurally similar to groups that had already been used to probe the N^6 and C2′ areas. The majority of top DOCK-ranked carboxylic acids were benzoic acid derivatives. Still, we attempted to select a diverse group of ligands while, at the same time, including top DOCK-ranked substituents known to enhance adenosine affinity (Table 4; 3-methylbenzyl-, 2-methylbenzyl-, naphthylenemethyl-, and benzylamine; 4-phenylbenzoic acid,[22] 3,5-dimethoxybenzoic acid, and 3,5-dichlorobenzoic acid). This led to a selection of 20 amines and 12 carboxylic acids (Table 4) that were used to generate a 240-compound library of *N*^{6}-(substituted)-2′-deoxy-2′-(amido)adenosine analogues via parallel solution-phase synthesis based on the common intermediate **5**. The solvation-corrected DOCK scores for the 1400 carboxylic acids ranged from 10.22 to 87.13 kcal/mol, while those for the 460 amines ranged from 14.17 to 1814.83 kcal/mol. The 12 acids and 20 amines selected for parallel synthesis were chosen from the top 200 scoring compounds of their respective virtual libraries for which the difference in force field scores between the best and the worst compounds was no more than 10 kcal/mol.

N^6-(Substituted)-2′-deoxy-2′-aminoadenosine intermediates were purified by preparative HPLC, and the

Table 3. Inhibition of *L. mexicana* GAPDH by *N*^{6}-(Subsutituted)-2′-deoxy-2′-(benzamido)adenosine Analogues



6aa and **6ab**
11e and **11n**
11m-18m

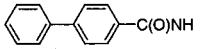
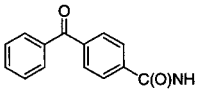
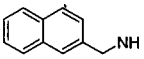
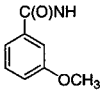
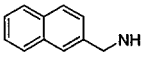
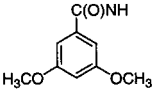
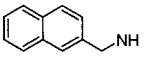
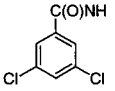
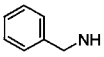
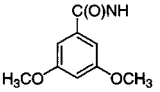
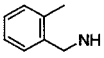
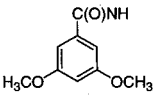
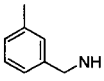
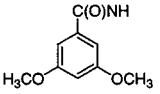
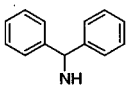
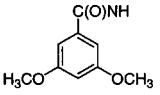
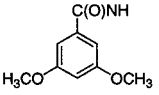
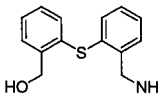
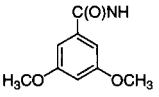
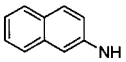
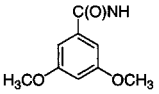
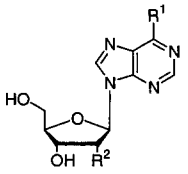
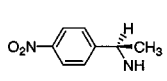
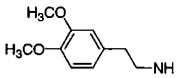
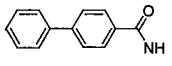
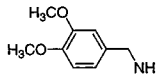
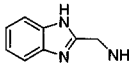
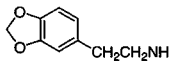
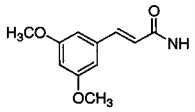
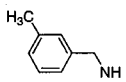
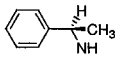
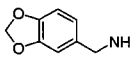
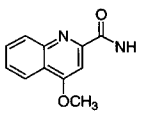
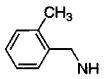
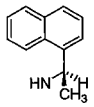
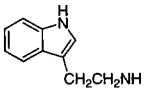
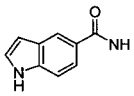
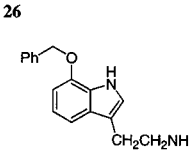
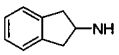
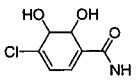
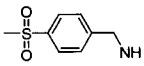
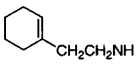
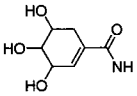
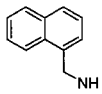
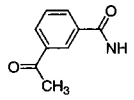
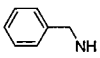
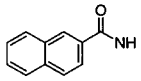
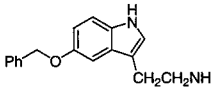
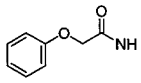
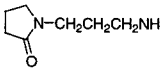
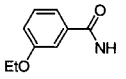
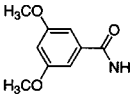
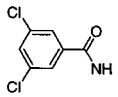
Compound	R^1	R^2	IC_{50} (μM)
6aa			inactive
6ab			inactive
11e			2
11m			2
11n			25
12m			20
13m			25
14m			25
15m			55
16m			75
17m			75
18m			60

Table 4. Primary Amines and Carboxylic Acids Selected from Docked ACD Answer Set[a]



6ac-6al
6m, 12-14m, and **19-34m**
6n, 12-14n, and **19-34n**
12-14ac - 12-14al
19ac-34al

R^1		R^2		R^1		R^2	
19		**27**		**ac**		**25**	
20		**28**		**ad**		**14**	
21		**29**		**ae**		**13**	
22		**30**		**af**		**26**	
23		**31**		**ag**			
24		**32**		**ah**			

R^1		R^2	
6		**ai**	
12		**aj**	
33		**ak**	
34		**al**	
		m	
		n	

[a] Substituents are presented in qualitative order of decreasing DOCK scores. For example, reading down the column, **19** > **20** > **14** > **26**.

final acylated library was spot-checked by ESI-MS and HPLC (as described in the Experimental Section) to ensure that the desired final products were present in reasonable yield. The crude library was screened against *L. mexicana* GAPDH at an approximate concentration of 30 μM to yield a number of low-micromolar inhibitors (Chart 1), but none were superior to **6m**. Compounds **6m**, **6af**, **28m**, **29m**, and **30m** were isolated from their respective crude reaction, characterized, and assayed against *L. mexicana* GAPDH to give IC_{50}'s of 2, 20, 20, 8, and 20 μM, respectively.

The relative experimental potencies of the library compounds did not correlate well with their solvation-corrected DOCK scores, but it should be noted that the scores for these 240 compounds were all among the very best scores (within 10 kcal/mol of each other) compared to the much larger range of energies for all compounds analyzed computationally, as noted above. Indeed, 25% of the 240 compounds display an IC_{50} that was within 4- to 15-fold of our best inhibitor, **6m**. Thus, the process of using DOCK provided approximately 40 new, reasonably potent GAPDH inhibitors. It is presumed that many of the poor-scoring compounds would be poor GAPDH inhibitors and thus were not synthesized. To put the DOCK program to a rigorous test, a large number of compounds with poor DOCK scores would need to be synthesized. We refrained from doing this because we believe that we have thoroughly explored 2′,N^6-disubstituted adenosines as parasite GAPDH inhibitors.

Modifications of the C5′ of N^6-(1-Naphthalenemethyl)adenosine and 2′-Deoxy-2′-(3,5-dimethoxybenzamido)adenosine (Table 5). Both 5′deoxy-(5′-cyclohexylacetamido)adenosine (**35**)[23] and 5′deoxy-(5′-diphenylacetamido)adenosine (**36**)[22] inhibit *L. mexicana* GAPDH with IC_{50}'s of 250 μM. We wished to investigate if the integration of these C5′ substituents with either the N^6-naphthalenemethyl or C2′-(3,5-dimethoxybenzamido) substituents would increase binding affinity. Surprisingly, N^6-(1-naphthalenemethyl)-5′deoxy-(5′-cy-

Chart 1. Screening of N^6-(Substituted)-2′-deoxy-2′-(amido)Adenosine Combinatorial Library against *L. mexicana* GAPDH

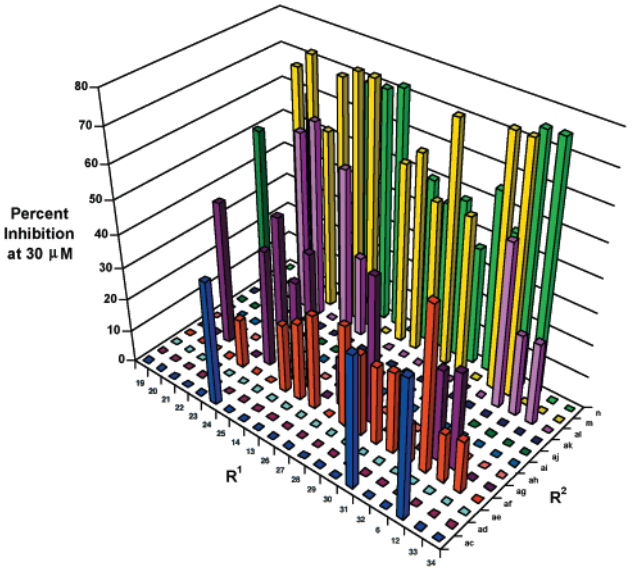
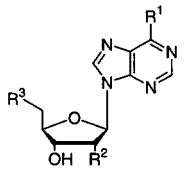
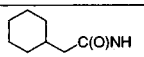
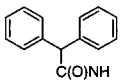
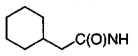
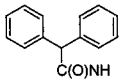
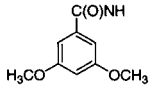
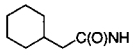
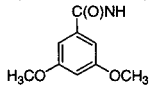
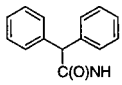


Table 5. Inhibition of *L. mexicana* GAPDH by 5′-Deoxy-5′-amido-, N^6-(Substituted)-5′-deoxy-5′-amido-, and 2′,5′-Dideoxy-2′,5′-bisamidoadenosine Analogues[a]



35, 36, 41, 42, 48, and 49

Compound	R^1	R^2	R^3	IC_{50} (μM)
35	NH_2	OH		250
36	NH_2	OH		250
41		OH		inactive
42		OH		inactive
48	NH_2			100
49	NH_2			60

[a] Inactive = inactive at 50 μM.

clohexylacetamido)adenosine (**41**) and N^6-(1-naphthalenemethyl)-5′deoxy-(5′-diphenylacetamido)adenosine (**42**) did not inhibit GAPDH when tested up to their solubility limit of 50 μM. 2′,5′-Dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(cyclohexylacetamido)adenosine (**48**) and 2′,5′-dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(diphenylacetamido)adenosine (**49**) did inhibit *L. mexicana* GAPDH with IC_{50}'s of 100 and 60 μM, respectively. Docking experiments positioned all four C5′-substituted compounds such that their adenosyl moieties and their respective N^6 or C2′ substituents were superimposed with the coordinates of **6m**. It is unclear why compounds **41** and **42** did not benefit from the additive effect that **48** and **49** did. Solubility limitations hindered a careful examination of the competitive and selective nature of **49**.

GAPDH Cross Species Evaluation and Trypanosomatid Growth Inhibition Studies (Table 6). Only the more potent compounds (**6e**, **6m**−**6q**, **7e**, **7f**, **7m**, **7n**, **9e**, **9m**, **9n**, **10e**, **11e**, **11m**, and **11n**) were screened against *T. brucei* and *T. cruzi* GAPDH (Table 5) because these two enzymes are more difficult to overexpress and purify, and only small quantities were available. In general, the activity of the adenosine analogues decreases in the order *L. mexicana*, *T. cruzi*, and *T. brucei* (Table 6). This was also true for 2′,5′-dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(diphenylacetamido)adenosine, **49**, which displayed IC_{50}'s of 60, 82, and 100 μM for the respective parasite GAPDH's. Although the homology between the adenosyl binding regions is highly conserved across trypanosomatids, the replacement of Ser-40 in the selectivity cleft of *L. mexicana* with an asparagine in *T. cruzi* and *T. brucei* may account for the small differences in affinity. All of these compounds were tested against rabbit muscle GAPDH (mammalian control) and were found to be completely selective for the parasite enzymes up to their solubility limit. Compound **6m** was shown to be competitive with NAD^+ (data not shown), which is consistent with the crystallographic data.

The above-mentioned inhibitors were tested for the ability to block the growth of bloodstream *T. brucei*, mammalian stage *T. cruzi* grown in murine 3T3 fibroblasts, and murine 3T3 fibroblasts (Table 6). All cell culture assays were performed twice with very similar results. Suprisingly, ED_{50}'s for the cultured parasites were within a 2- to 4-fold range of the IC_{50}'s measured against GADPH in vitro, with the exception of **6n**. On the basis of the aforementioned computational studies performed by Baker et al.,[12−14] a 10- to 100-fold difference between IC_{50}'s and EC_{50}'s might be expected. It is possible that these analogues accumulate in parasites to a higher concentration than present extracellularly. Alternatively, it is possible these compounds act on a target other than or in addition to GAPDH. The ED_{50}'s of the compounds against mammalian cells were consistently higher than the ED_{50}'s against parasites. This is consistent with the selectivity of the adenosine analogues for parasitic GAPDH over mammalian.

Conclusions

New robust routes for the synthesis of N^6-(substituted)-2′-deoxy-2′-(amido)adenosine and N^6-(substituted)-5′-deoxy-5′-(amido)adenosine analogues and a route to a new class of derivative, 2′,5′-dideoxy-2′,5′-(bisamido)-adenosine, have been developed. The synthetic route from the common intermediate 9-[2′-deoxy-2′-trifluoroacetamido-3′,5′-O-(diacetyl)]-(1-β-D-ribofuranosyl)-6-chloropurine (**5**) allows a library of hundreds of N^6-(substituted)-2′-deoxy-2′-(amido)andenosines to be readily synthesized via parallel solution-phase synthesis. Utilizing structure-based design methodology, we synthesized a number of inhibitors that are 7000- to 25000-fold more active (across trypanosmatid species) than

Table 6. Effect of Adenosine Analogues on Trypanosomatide GAPDH and on Growth of Cultured Parasites

compd	*L. mexicana* IC_{50} (μM)	*T. brucei* IC_{50} (μM)	*T. brucei* ED_{50} (μM)	*T. cruzi* IC_{50} (μM)	*T. cruzi* ED_{50} (μM)	rabbit muscle IC_{50} (μM)	3T3 fibroblasts ED_{50} (μM)
6e	6	25	18, 16	12	10, 12	>50	40, 50
6m	2	7	17, 17	7	8, 13	>50	40, 37
6n	12	100	5, 4	75	7, 7	>50	35, 33
6o	10	14	>50, >50	12	>50, >50	>50	>50, >50
6p	5	26	50, 35	14	>50, >50	>50	>50, >50
6q	4	47	36, 40	7	22, 28	>50	>55, 44
7e	6	25	35, 34	12	>50, >50	>50	>50, >50
7f	4	25	16, 20	12	30, 48	>50	>50, >50
7m	2	8	38, 38	10	>50	>50	>50, >50
7n	25	55	12, 20	40	41, 38	>50	43, 50
9e	2	12	31, 32	12	20, 33	>50	>50, >50
9m	2	5	35, 40	7	35, 30	>50	50, >50
9n	25	45	16, 16	40	16, 16	>50	32, 38
10e	5	26	10, 12	50	12, 12	>50	50, 50
11e	2	24	19, 36	10	10, 17	>50	>50, >50
11m	2	10	37, 35	5	25, 21	>50	50, >50
11n	25	30	16, 17	30	15, 13	>50	34, 38
pentamidine	*a*	*a*	0.006, 0.004	*a*	*a*	>50	*a*
benznidazole	*a*	*a*	*a*	*a*	0.5, 0.5	>50	>50, >50

[a] Not determined.

adenosine. Additionally, the application of rational design and combinatorial chemistry led to a number of low-micromolar inhibitors of *L. mexicana* GAPDH. The most potent GAPDH inhibitors block the growth of bloodstream *T. brucei* and the intracellular stage *T. cruzi* in the low-micromolar range.

Experimental Section

General Synthetic Procedures. CH_2Cl_2 was freshly distilled over CaH_2 under argon. 6-Chloropurine-9-riboside and anhydrous DMF were purchased from Aldrich Chemical Co. Ltd. 4-Sulfonamidobutyryl-AM resin (loading, 0.4−1.2 mmol/g; polymer matrix, copoly(styrene−1% DVB); 200−400 mesh) was purchased from Novabiochem. ^{1}H NMR spectra were recorded on a Bruker AF 300 MHz or AM 500 MHz spectrometer in DMSO-d_6. Electrospray ionization mass spectra (EIS-MS) were obtained on a Bruker Esquire LC ion trap or Kratos Profile HV4 mass spectrometer. Reactions were monitored by TLC on precoated silica gel 60 F_{254} glass plates (EM Science) with CH_2Cl_2/EtOH (9:1) or $CHCl_3$/MeOH (80:20) as eluent and visualized at 254 nm. Medium-pressure flash column chromatography was carried out on silica gel 60 (230−400 mesh; EM Science) with $CHCl_3$/MeOH (98:2 to 80:20) eluent. Preparative HPLC was performed on a C_{18} column (Vydac 218TP1010) using one of the following elution gradients: (method A) linear gradient of 10 mM triethylammonium acetate (aq, pH = 7.0) from 0 to 100% ACN over 60 min at 4.0 mL/min; (method B) linear gradient of 5% HCOOH (aq) from 0 to 100% MeOH over 60 min at 4.0 mL/min; (method C) isocratic 10 mM triethylammonium acetate (aq, pH = 7.0) of 0% over 15 min, then a linear gradient of 10 mM triethylammonium acetate (aq, pH = 7.0) from 0 to 25% ACN over 45 min at 4.0 mL/min; (method D) same as method B except a flow rate of 7.0 mL/min on a C_{18} column (Vydac 218TP1022) was used. The purity of all compounds was verified by HPLC with methods A and B on a C_{18} column (Vydac 218TP52) at 1.0 mL/min.

Synthesis of 2′-Amino-2′-deoxyadenosine (1). Compound **1** was synthesized via 2′-azido-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine as per the reliable methodologies of Robins et al.[26] and Van Calenberg et al.[20] Because minor modifications to the procedure were made that resulted in slightly improved yields, we have included the details of the preparation in Supporting Information.

Synthesis of 2′-Amino-2′-deoxyinosine (2). Starting with **1** (4.0 g, 15.0 mmol), the procedure of Kotra et al..[31] was used to give 4.0 g of **2** (99.0%). ^{1}H NMR: δ 3.54 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.95 (m, 2H, C3′-H and C4′-H), 4.18 (m, 1H, C2′-H), 5.64 (d, 1H, C1′-H), 8.10 (d, 1H, C2-H), 8.27 (s, 1H, C8-H), 12.51 (s, 1H, N1-H). ESI-MS: *m*/*z* 268.1 $(M + H^+)^+$.

Synthesis of 2′-Deoxy-2′-trifluoroacetamidoinosine (3). Ethyl trifluoroacetate (5.36 mL, 45.0 mmol) was added to a solution of **2** (4.0 g, 15.0 mmol) in anhydrous DMF (50 mL), and the reaction was heated at 60 °C under argon for 3 h. The reaction was evaporated via rotary evaporation at 50 °C and then further dried in vacuo for 24 h. The resulting amorphous solid was of sufficient purity to use in the subsequent reaction. For characterization, a small sample was purified via flash chromatography. ^{1}H NMR: δ 3.57 and 3.68 (2 × m, 2H, C5′-$H_{a,b}$), 4.03 (m, 1H, C4′-H), 4.34 (m, 1H, C3′-H), 5.02 (m, 1H, C2′-H), 5.19 (br s, 1H, C5′-O*H*), 5.92 (br s, 1H, C3′-O*H*), 6.18 (d, 1H, C1′-H), 8.10 and 8.27 (2 × s, 2H, C2-H and C8-H), 9.65 (d, 1H, C2′−N*H*), 12.54 (s, 1H, N1-H). ESI-MS: *m*/*z* 364.1 $(M + H^+)^+$.

Synthesis of 2′-Deoxy-2′-trifluoroacetamido-3′,5′-O-(diacetyl)inosine (4). To a mixture of **3** (4.5 g, ∼12.4 mmol) in anhydrous ACN (250 mL) was added sequentially Ac_2O (11.7 mL, 124 mmol), anhydrous Et_3N (13.8 mL, 99.2 mmol), and DMAP (128 mg, 1.0 mmol) at ambient temperature under argon. After the mixture was stirred for 1 h at ambient temperature, MeOH (15.0 mL) was added and then the reaction was evaporated via rotary evaporation at 40 °C. The resulting residue was partitioned between EtOAc (250 mL) and H_2O (250 mL), washed with H_2O (2 × 250 mL), washed with saturated NaCl (aq, 2 × 250 mL), dried over $MgSO_4$, filtered, and then evaporated. The crude material was purified via flash chromatography to yield 6.2 g of **4** (93% based on **2** as the starting material). ^{1}H NMR: δ 2.04 (s, 3H, C5′-OAc), 2.08 (s, 3H, C3′-OAc), 4.23 (m, 1H, C5′-H_a), 4.38 (m, 2H, C4′-H and C5′-H_b), 5.37 (m, 1H, C3′-H), 5.44 (m, 1H, C2′-H), 6.23 (d, 1H, C1′-H), 8.10 and 8.34 (2 × s, 1H, C2-H and C8-H), 10.10 (br s, 1H, C2′-N*H*), 12.50 (s, 1H, N1-H). ESI-MS: *m*/*z* 448.1 $(M + H^+)^+$.

Synthesis of 9-[2′-Deoxy-2′-trifluoroacetamido-3′,5′-O-(diacetyl)]-(1-β-D-ribofuranosyl)-6-chloropurine (5). With minor modifications, the methodology of Matsuda et al.[32] was employed. To a rapidly stirring mixture of **4** (6.0 g, 13.4 mmol), Et_4NCl (8.9 g, 53.7 mmol), and dry *N*,*N*-dimethylaniline (1.7 mL, 13.4 mmol) in anhydrous ACN (30 mL) was added $POCl_3$ (3.1 mL, 33.6 mmol) at ambient temperature under argon. The reaction was placed in a preheated oil bath and refluxed at 100 °C for 10 min. The reaction was then evaporated via rotary evaporation at 40 °C, and the resulting green foam was dissolved in CH_2Cl_2 (100 mL) and then stirred vigorously over crushed ice for 15 min. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phase was then washed with H_2O (2 × 100

mL), washed with 5% $NaHCO_3$ (aq, pH ~7, 2 × 100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting yellow foam was then purified via flash chromatography to give 5.7 g of **5** as a white foam (92.0%). ^{1}H NMR: δ 2.15 (s, 3H, C5′-OAc), 2.20 (s, 3H, C3′-OAc), 4.28 and 4.41 (2 × m, 2H, C5′-$H_{a,b}$), 4.44 (m, 1H, C4′-H), 5.50 (m, 2H, C2′-H and C3′-H), 6.42 (d, 1H, C1′-H), 8.87 and 8.95 (2 × s, 1H, C2-H and C8-H), 10.09 (d, 1H, C2′-N*H*). ESI-MS: *m*/*z* 448.1 $(M + H^+)^+$. ESI-MS: *m*/*z* 466.1 $(M + H^+)^+$.

General Procedure for the Preparation of N^6-(Substituted)-2′-Deoxy-2′-aminoadenosine Analogues (6−18). To a solution of **5** (10.0 mg, 2.2 × 10^{-2} mmol) in EtOH (500 μL), in a 4.0 mL screw cap vial with Teflon septum, was added the appropriate amine or amine hydrochloride (6.4 × 10^{-2} mmol) and then Et_3N (9.0 μL, 6.4 × 10^{-2} mmol for free amines; 18.0 μL, 12.8 × 10^{-2} mmol for amine hydrochlorides). The reaction was heated at 60 °C for 18 h and then cooled to ambient temperature. Concentrated NH_4OH (aq, 500 μL) was then added, and the reaction was heated at 80 °C for 24 h. Without further workup, the reaction was purified via preparative HPLC using method A. The desired fraction was concentrated and then lyophilized to obtain the desired products in 85−95% yield.

***N*^{6}-(1-Naphthalenemethyl)-2′-deoxy-2′-aminoadenosine (6).** ^{1}H NMR: δ 3.53 and 3.64 (2 × m, 2H, C5′-$H_{a,b}$), 3.98 (m, 3H, C2′-H, C3′-H, and C4′-H), 5.19 (br s, 3H, N^6-CH_2 and C5′-O*H*), 5.50 (br s, 1H, C3′-O*H*), 5.68 (d, 1H, C1′-H), 7.42 (m, 2H, naphthyl-H), 7.55 (m, 2H, naphthyl-H), 7.81 (d, 1H, naphthyl-H), 7.94 (d, 1H, naphthyl-H), 8.15 and 8.36 (2 × s, 2H, C2-H and C8-H), 8.19 (d, 1H, naphthyl-H), 8.50 (br s, 1H, N^6-H). ESI-MS (MeOH): *m*/*z* 407.2 $(M + H)^+$.

General Procedure for the Preparation of N^6-(Substituted)-2′-deoxy-2′-(amido)adenosine Analogues. EDCI (3.7 × 10^{-2} mmol) was added to a solution of the appropriate carboxylic acid (3.7 × 10^{-2} mmol) in anhydrous CH_2Cl_2 (100 μL), and the solution was stirred under argon for 30 min. A total of 100 μL of this solution was added to a solution of N^6-(substituted)-2′-deoxy-2′-aminoadenosine (1.2 × 10^{-2} mmol) in anhydrous DMF (100 μL), and the mixture was stirred for 30 min. Without further workup, the reaction mixture was purified via preparative HPLC using method B to obtain the desired products in 85−95% yields after removal of solvents. For reactions involving acetylated hydroxybenzoic acids, the solutions were evaporated to dryness. The resulting solid was dissolved in MeOH (250 μL) and cooled to 0 °C, and then NH_3-(g) was bubbled into the solution. The reaction mixture was stirred at ambient temperature for 15 min and then, without further workup, was purified by preparative HPLC using method B.

***N*^{6}-(1-Naphthalenemethyl)-2′-deoxy-2′-(3,5-dimethoxybenzamido)adenosine (6m).** ^{1}H NMR: δ 3.61 and 3.72 (2 × m, 2H, C5′-$H_{a,b}$), 3.75 (s, 6H, OCH_3), 4.10 (m, 1H, C4′-H), 4.33 (m, 1H, C3′-H), 5.16 (br s, 2H, N^6-CH_2), 5.34 (m, 1H, C2′-H), 5.60 (br s, 1H, C5′-O*H*), 5.79 (br s, 1H, C3′-O*H*), 6.23 (d, 1H, C1′-H), 6.63 (s, 1H, Ph-H), 6.98 (s, 2H, Ph-H), 7.39 (m, 2H, naphthyl-H), 7.52 (m, 2H, naphthyl-H), 7.80 (d, 1H, naphthyl-H), 7.94 (d, 1H, naphthyl-H), 8.21 and 8.31 (2 × s, 2H, C2-H and C8-H), 8.22 (d, 1H, naphthyl-H), 8.42 (d, 1H, C2′-N*H*), 8.55 (br s, 1H, N^6-H). ESI-MS: (MeOH) *m*/*z* 571.2 $(M + H)^+$.

Combinatorial Library. The 240 N^6-(substituted)-2′-deoxy-2′-(amido)adenosine derivatives were synthesized individually via solution-phase parallel synthesis. Starting with **5** (50 mg for each amine reaction, 0.11 μmol), the above procedure for the synthesis of N^6-substituted-2′-deoxy-2′-aminoadenosine analogues was followed using the set of 20 amines shown in Table 4. These intermediates were purified by preparative HPLC using method D and characterized by NMR and ESI-MS (see Supporting Information) to yield the desired intermediates in 85−95% yield. Acylation of the 2′-aminoadenosine derivatives (approximately 4.4 μmol each) was accomplished as described above, and the reaction progress was monitored by TLC. Two members from each carboxylic acid vector were chosen (such that each amine vector was represented at least once) from the 20 × 12 matrix and were analyzed by ESI-MS and HPLC. For carboxylic acid vectors **ac**, **ad**, **ai**, **aj**, **ak**, **al**, **m**, and **n** (Table 4), acylation reactions gave 80−95% conversion to the desired products while **ae** and **af** gave approximately 50% and **ag** and **ah** gave approximately 25%. Upon completion of the reaction, the solutions were diluted with MeOH and then evaporated to dryness in a Speed-Vac. The crude material was dissolved in DMSO and used for in vitro enzyme inhibition analysis.

6-Chloropurine-5′-deoxy-(5′-phthalamido)riboside (38). To a solution of 6-chloropurine-9-riboside (**37**, 1.0 g, 3.5 mmol), phthalimide (615 mg, 4.2 mmol), and triphenylphosphine (1.8 g, 7.0 mmol) in anhydrous THF (10 mL) was added DIAD (1.3 mL, 7.0 mmol) at ambient temperature under argon. After the mixture was stirred for 2 h, MeOH (10 mL) was added to the reaction mixture that was then stirred for an additional 15 min. Without further workup, the reaction was absorbed onto silica gel and purified via flash chromatography to give 1.2 g of **38** (86.0%). ^{1}H NMR: δ 3.98 (m, 2H, C5′-H), 4.18 (m, 1H, C4′-H), 4.25 (m, 1H, C3′-H), 4.80 (m, 1H, C2′-H), 5.41 (d, 1H, O*H*), 5.62 (d, 1H, O*H*), 6.01 (s, 1H, C1′-H), 7.82 (s, 4H, phthalimide-H), 8.59 and 8.96 (2 × s, 2H, C2-H and C8-H). ESI-MS: *m*/*z* 416.1 $(M + H^+)^+$.

***N*^{6}-(1-Naphthalenemethyl)-5′deoxy-5′-aminoadenosine (40).** To a solution of **38** (100 mg, 2.4 × 10^{-1} mmol) and Et_3N (101 μL, 7.2 × 10^{-1} mmol) in EtOH (5 mL) was added 1-naphthalenemethylamine (113 μL, 7.2 × 10^{-1} mmol). After the solution was refluxed for 18 h, conversion to **39** was complete, as determined by TLC and ESI-MS, and the sample was used without further workup. Hydrazine hydrate (50% aq, 82 μL, 2.4 mmol) was then added to the crude reaction mixture and was refluxed for 18 h. Without further workup the reaction was purified via preparative HPLC method A to give 91 mg of **40** (93.6%). ^{1}H NMR: δ 2.79 and 2.87 (2 × m, 2H, C5′-$H_{a,b}$), 3.92 (m, 1H, C4′-H), 4.15 (m, 1H, C3′-H), 4.69 (m, 1H, C2′-H), 5.17 (br s, 3H, N^6-CH_2), 5.87 (d, 1H, C1′-H), 7.41 (m, 2H, naphthyl-H), 7.55 (m, 2H, naphthyl-H), 7.80 (d, 1H, naphthyl-H), 7.94 (d, 1H, naphthyl-H), 8.17 and 8.41 (2 × s, 2H, C2-H and C8-H), 8.22 (d, 1H, naphthyl-H), 8.44 (br s, 1H, N^6-H). ESI-MS (MeOH): *m*/*z* 407.2 $(M + H)^+$.

General Procedure for the Preparation of N^6-(1-Naphthalenemethyl)-5′-deoxy-5′-(amido)adenosine Analogues. Method A. EDCI (3.7 × 10^{-2} mmol) was added to a solution of the appropriate carboxylic acid (3.7 × 10^{-2} mmol) in anhydrous CH_2Cl_2 (100 μL), and the mixture was stirred under argon for 30 min. A total of 100 μL of this solution was added to a solution of **40** (5.0 mg, 1.2 × 10^{-2} mmol) in anhydrous DMF (100 μL), and the reaction mixture was stirred for 30 min. Without further workup, the reaction mixture was purified via preparative HPLC using method B to obtain the desired products **41** and **42** in 75 and 90% yields, respectively.

Method B. A mixture of the appropriately acylated safety-catch resin (6.0 × 10^{-2} mmol) and **40** (5.0 mg, 1.2 × 10^{-2} mmol) was stirred under argon in anhydrous DMF (500 μL) for 18 h at ambient temperature. The product was filtered from the resin, and the resin was washed with DMF (500 μL), CH_2Cl_2/MeOH (1:1, 2 × 500 μL), and CH_2Cl_2 (2 × 500 μL). The filtrate and washings were combined and then evaporated to dryness via rotary evaporation at 40 °C. The desired products were obtained in >95% yield and purity as determined by HPLC. ^{1}H NMR and ESI-MS of samples prepared via methods A and B were identical.

***N*^{6}-(1-Naphthalenemethyl)-5′deoxy-(5′-cyclohexylacetamido)adenosine (41).** ^{1}H NMR: δ 0.85−1.65 (band, 11H, cyclohexyl-H), 1.99 (d, 2H, C5′-NHC(O)CH_2), 3.35 and 3.45 (2 × m, 2H, C5′-$H_{a,b}$), 3.95 (m, 1H, C4′-H), 4.01 (m, 1H, C3′-H), 4.69 (m, 1H, C2′-H), 5.18 (br s, 2H, N^6-CH_2), 5.28 (br s, 1H, O*H*), 5.49 (br s, 1H, O*H*), 5.85 (d, 1H, C1′-H), 7.41 (m, 2H, naphthyl-H), 7.55 (m, 2H, naphthyl-H), 7.80 (d, 1H, naphthyl-H), 7.94 (d, 1H, naphthyl-H), 8.12 (m, 1H, C5′-N*H*), 8.20 and 8.35 (2 × s, 2H, C2-H and C8-H), 8.22 (d, 1H, naphthyl-H), 8.44 (br s, 1H, N^6-H). ESI-MS: *m*/*z* 531.3 $(M + H^+)^+$.

N^6-(1-Naphthalenemethyl)-5′deoxy-(5′-diphenylacetamido)adenosine (42). ^{1}H NMR: δ 3.42 and 3.53 (2 × m, 2H, C5′-$H_{a,b}$), 3.95 (m, 1H, C4′-H), 4.01 (m, 1H, C3′-H), 4.64 (m, 1H, C2′-H), 4.98 (s, 1H, C5′-NHC(O)C*H*), 5.18 (br s, 1H, N^6-C*H*), 5.28 (br s, 1H, O*H*), 5.46 (br s, 1H, O*H*), 5.85 (d, 1H, C1′-H), 7.17−7.32 (band, 10H, Ph-H), 7.41 (m, 2H, naphthyl-H), 7.55 (m, 2H, naphthyl-H), 7.80 (d, 1H, naphthyl-H), 7.94 (d, 1H, naphthyl-H), 8.20 and 8.35 (2 × s, 2H, C2-H and C8-H), 8.21 (d, 1H, naphthyl-H), 8.44 (br s, 1H, N^6-H), 8.57 (m, 1H, C5′-N*H*). ESI-MS: *m*/*z* 601.3 $(M + H^+)^+$.

2′-Deoxy-2′-trifluoroacetamidoadenosine (43). Ethyl trifluoroacetate (135 μL, 1.1 mmol) was added to a solution of **1** (100 mg, 3.7×10^{-1} mmol) in anhydrous DMF (1.25 mL), and the reaction mixture was heated at 60 °C under argon for 3 h. The reaction mixture was evaporated via rotary evaporation at 50 °C and then further dried in vacuo for 24 h. The resulting amorphous solid was purified via flash chromatography to yield 130 mg of **43** (95.5%). ^{1}H NMR: δ 3.57 and 3.68 (2 × m, 2H, C5′-$H_{a,b}$), 4.05 (m, 1H, C4′-H), 4.35 (m, 1H, C3′-H), 5.12 (m, 1H, C2′-H), 5.57 (m, 1H, C5′-O*H*), 5.84 (d, 1H, C3′-O*H*), 6.21 (d, 1H, C1′-H), 7.41 (br s, 2H, N^6-H), 8.13 and 8.24 (2 × s, 2H, C2-H and C8-H), 9.60 (br s, 1H, C2′-N*H*). ESI-MS: *m*/*z* 363.1 $(M + H^+)^+$.

2′,5′-Dideoxy-2′-(trifluoroacetamido)-5′-phthalimidoadenosine (44). To a solution of **43** (100 mg, 2.8×10^{-1} mmol), phthalimide (49.4 mg, 3.4×10^{-1} mmol), and triphenylphosphine (144 mg, 5.5×10^{-1} mmol) in anhydrous THF (1.0 mL) was added DIAD (80.5 μL, 5.5×10^{-1} mmol) at ambient temperature under argon. After the solution was stirred for 2 h, MeOH was added to the reaction mixture (1.0 mL), which was then stirred for an additional 15 min. Without further workup, the reaction was absorbed onto silica gel and purified via flash chromatography to give 111 mg of **44** (81.5%). ^{1}H NMR: δ 3.85 and 4.02 (2 × dd, 2H, C5′-$H_{a,b}$), 4.22 (m, 1H, C4′-H), 4.49 (m, 1H, C3′-H), 5.28 (t, 1H, C2′-H), 5.91 (br s, 1H, C3′-O*H*), 6.12 (d, 1H, C1′-H), 7.31 (br s, 2H, N^6-H), 7.84 (s, 4H, phthalimide-H), 7.91 and 8.34 (2 × s, 2H, C2-H and C8-H), 9.60 (d, 1H, C2′-N*H*). ESI-MS: *m*/*z* 492.1 $(M + H^+)^+$.

2′,5′-Dideoxy-2′,5′-(bisamino)adenosine (45). A solution of **44** (100 mg, 2.0×10^{-1} mmol) and hydrazine hydrate (50% aq, 94 μL, 2.8 mmol) in EtOH (5.0 mL) was refluxed for 18 h. Without any further workup, the crude reaction mixture was purified via preparative HPLC method C to yield 48 mg of **45** (88.9%). ^{1}H NMR: δ 2.88 (m, 2H, C5′-H), 3.95 (m, 1H, C4′-H), 4.02 (m, 2H, C2′-H and C3′-H), 5.65 (d, 1H, C1′-H), 7.23 (br s, 2H, N^6-H), 8.12 and 8.32 (2 × s, 2H, C2-H and C8-H). ESI-MS: *m*/*z* 266.2 $(M + H^+)^+$.

General Procedure for the Preparation of 2′,5′-Dideoxy-2′-amino-5′-(amido)adenosine Analogues. See Method B of General Procedure for the Preparation of N^6-(1-Naphthalenemethyl)-5′-deoxy-5′-(Amido)adenosine Analogues.

2′,5′-Dideoxy-2′-amino-5′-(cyclohexylacetamido)adenosine (46). ^{1}H NMR: δ 0.87−1.65 (band, 11H, cyclohexyl-H), 2.04 (d, 2H, C5′-NHC(O)CH_2), 3.38 and 3.51 (2 × m, 2H, C5′-$H_{a,b}$), 3.87 (m, 1H, C4′-H), 3.96 (m, 2H, C2′-H and C3′-H), 5.61 (d, 1H, C1′-H), 7.33 (br s, 2H, N^6-H), 8.14 and 8.29 (2 × s, 3H, C2-H, C8-H, and C5′-N*H*). ESI-MS: *m*/*z* 390.2 $(M + H^+)^+$.

2′,5′-Dideoxy-2′-amino-5′-(diphenylacetamido)adenosine (47). ^{1}H NMR: δ 3.38 and 3.51 (2 × m, 2H, C5′-$H_{a,b}$), 3.82 (m, 2H, C3′-H and C4′-H), 3.89 (m, 1H, C2′-H), 5.02 (s, 1H, N^6-CH), 5.61 (d, 1H, C1′-H), 7.18−7.32 (band, 12H, N^6-H and Ph-H), 8.16 and 8.25 (2 × s, 2H, C2-H and C8-H), 8.75 (m, 1H, C5′-N*H*). ESI-MS: *m*/*z* 460.2 $(M + H^+)^+$.

General Procedure for the Preparation of 2′,5′-Dideoxy-2′-amido-5′-(amido)adenosine Analogues. See Method A of General Procedure for the Preparation of N^6-(1-Naphthalenemethyl)-5′-deoxy-5′-(amido)adenosine Analogues. The desired products **48** and **49** were obtained in 86 and 92% yields, respectively.

Method B. As per the methodology of Link et al.,[33] a mixture of the activated 3,5-dimethoxybenzylsulfonamide safety-catch resin (6.0×10^{-2} mmol) and **46** or **47** (1.2×10^{-2} mmol) was stirred under argon in anhydrous DMF (500 μL) for 6 h at 60 °C. The product was filtered from the resin, and the resin was washed with DMF (500 μL), CH_2Cl_2/MeOH (1:1, 2 × 500 μL), and CH_2Cl_2 (2 × 500 μL). The filtrate and washings were combined and then evaporated to dryness via rotary evaporation at 40 °C to give **48** and **49** in >90% yield and in >95% purity as determined by HPLC. ^{1}H NMR and ESI-MS of samples prepared via methods A and B were identical.

2′,5′-Dideoxy-2′-(3,5-dimethoxybenzamido)-5′-(cyclohexylacetamido)adenosine (48). ^{1}H NMR: δ 0.89−1.65 (band, 11H, cyclohexyl-H), 2.04 (d, 2H, C5′-NHC(O)CH_2), 3.58 (m, 2H, C5′-H), 3.78 (s, 6H, OCH_3), 4.10 (m, 1H, C4′-H), 4.19 (m, 1H, C3′-H), 5.34 (m, 1H, C2′-H), 5.92 (br s, 1H, O*H*), 6.17 (d, 1H, C1′-H), 6.64 (s, 1H, Ph-H), 6.98 (s, 2H, Ph-H), 7.39 (br s, 2H, N^6-H), 8.19 and 8.25 (2 × s, 2H, C2-H and C8-H), 8.39 (m, 1H, C5′-N*H*), 8.42 (d, 1H, C2′-N*H*). ESI-MS: *m*/*z* 554.3 $(M + H^+)^+$.

2′,5′-Dideoxy-2′-(3″,5″-dimethoxybenzamido)-5′-(diphenylacetamido)adenosine (49). ^{1}H NMR: δ 3.58 (m, 2H, C5′-H), 3.75 (s, 6H, OCH_3), 4.07 (m, 1H, C4′-H), 4.23 (m, 2H, C3′-H), 5.03 (s, 1H, C5′-NHC(O)C*H*), 5.37 (m, 1H, C2′-H), 5.92 (br s, 1H, O*H*), 6.14 (d, 1H, C1′-H), 6.62 (s, 1H, Ph-H), 6.96 (s, 2H, Ph-H), 7.19−7.33 (band, 12H, N^6-H and Ph-H), 8.09 and 8.26 (2 × s, 2H, C2-H and C8-H), 8.43 (d, 1H, C2′-N*H*), 8.73 (m, 1H, C5′-N*H*). ESI-MS: *m*/*z* 624.3 $(M + H^+)^+$.

Preparation of Acetylated Hydroxybenzoic Acids. The method of White et al. was employed.[39]

Preparation of Cyclohexylmethyl- and Diphenylmethylsulfonamidobutyryl-AM resin. These resins were prepared from sulfonamidobutyryl-AM resin as per the methodology of Kenner et al.[29] and Backes et al.[30]

General Procedure for the Synthesis of Naphthalenemethylamines from Naphthylnitriles. $LiAlH_4$ (2.7 mmol) was added to naphthylnitrile (2.7 mmol) in anhydrous THF (10.0 mL) at 0 °C, and the mixture was stirred for 1 h. The reaction was quenched with MeOH and then washed with H_2O (2 × 10 mL). The organic layer was then extracted with 1 N HCl (2 × 10 mL), and the combined extracts were left to stand for 24 h at 4 °C to yield crystals of the desired amine hydrochloride in 60−80% yield.

2-Naphthalenemethylamine Hydrochloride (49). ^{1}H NMR: δ 4.18 (br s, 1H, C2−CH_2), 7.55 (m, 2H), 7.62 (d, 1H), 7.89−7.99 (band, 4H), 8.46 (br s, 3H, NH_3). ESI-MS: *m*/*z* 158.1 $(M + H^+)^+$.

1-(Aminomethyl)-4-methoxynaphthalene Hydrochloride (53). ^{1}H NMR: δ 3.98 (s, 3H, OCH_3), 4.41 (br s, 2H, C1−CH_2), 7.01 (d, 1H), 7.57 (m, 2H), 7.65 (t, 1H), 8.09 (d, 1H), 8.23 (d, 1H), 8.41 (br s, 3H, NH_3). ESI-MS: *m*/*z* 188.1 $(M + H^+)^+$.

1-(Aminomethyl)-3-methoxynaphthalene Hydrochloride (55). 3-Methoxy-1-naphthonitrile was synthesized as described by DeCosta et al.[36] ^{1}H NMR: δ 3.89 (s, 3H, OCH_3), 4.49 (d, 2H, C1-CH_2), 7.32 (s, 1H), 7.38 (s, 1H), 7.45 (t, 1H), 7.53 (t, 1H), 7.89 (d, 1H), 8.02 (d, 1H), 8.42 (br s, 3H, NH_3). ESI-MS: *m*/*z* 188.1 $(M + H^+)^+$.

1-(Aminomethyl)-2-methoxynaphthalene Hydrochloride (51). To a solution of 2-methoxynaphthalene (500 mg, 3.2 mmol) in EtOH (2.5 mL) was added concentrated HCl (125 μL) and 2-chloro-*N*-(hydroxymethyl)acetamide (395 mg, 3.2 mmol). The reaction mixture was refluxed for 30 min and then was cooled to ambient temperature. Additional concentrated HCl (1.25 mL) was added, and the reaction mixture was refluxed for 1.5 h and then was cooled to ambient temperature. The desired product crystallized upon cooling to give 375 mg of **34** (53.0%). ^{1}H NMR: δ 4.06 (s, 3H, OCH_3), 4.38 (d, 2H, C1−CH_2), 7.34−7.60 (band, 3H), 7.92 (d, 2H), 8.06 (d, 1H), 8.11 (d, 1H), 8.38 (br s, 3H, NH_3). ESI-MS: *m*/*z* 188.1 $(M + H^+)^+$.

3-Hydroxy-1-naphthalenemethylamine Hydrobromide (56). To a solution of **41** (200 mg, 0.89 mmol) in CH_2Cl_2 (2.0 mL) was added BBr_3 (422 μL, 4.5 mmol) at ambient temperature. The reaction was refluxed for 1 h, cooled to ambient temperature, and then evaporated to dryness via rotary evaporation at 40 °C. The resulting solid was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL). The organic layer was washed with H_2O (3 × 25 mL), and the aqueous layers were combined. The aqueous layer was reduced in volume to

approximately 25 mL and was left to stand at 4 °C for 24 h to yield crystals of **56** (71%). ^{1}H NMR: δ 4.46 (d, 2H, C1−CH_2), 7.18 (s, 1H), 7.20 (s, 1H), 7.37 (t, 1H), 7.46 (t, 1H), 7.75 (d, 1H), 7.98 (d, 1H), 8.28 (br s, 3H, NH_3), 9.97 (s, 1H, O*H*). ESI-MS: *m*/*z* 174.1 $(M + H^+)^+$.

Inhibition Studies. Assays were performed at 23 °C in a final volume of 0.50 mL of a 0.10 M triethanolamine/HCl (pH = 7.5) assay buffer while the absorption at 340 nm was monitored using a quartz cuvette. Activity for GAPDH was measured in the direction of NADH formation. Fresh glyceraldehyde-3-phosphate was prepared from its diethylacetal [di-(cyclohexylammonium) salt] as described by the manufacturer (Sigma). All compounds tested were dissolved in DMSO-d_6 (the combinatorial library was dissolved in DMSO), and the final concentration of DMSO-d_6 in the assay was kept at 5%. The reaction was initiated by addition of enzyme, and control reactions were run in the absence of inhibitors but in the presence of 5% DMSO-d_6. Remaining activity was calculated as a percent of control using the initial velocities measured from 0 to 2 min. For compounds with IC_{50}'s less than or equal to 50 μM, inhibitor concentrations in the cuvette were varied, with at least five different concentrations used to determine the IC_{50} values. Statistical error limits on the IC_{50} values have been calculated and amount to 10% or less. For compounds with IC_{50}'s greater than 50 μM, these values were extrapolated from at least three different concentrations. Final concentration of the components in the assay buffer was as follows: 5.0 mM $MgSO_4$, 1.0 mM EDTA, 12.8 mM KH_2PO_4, 1.0 mM DTT, 1.0 mM NaN_3, 0.45 mM NAD^+ (Sigma), and 1.87 mM glyceraldehyde-3-phosphate. Trypanosome and Leishmania GAPDH were obtained as described previously.[24]

Parasite Cultures and Drug Screening Assays. *T. brucei* (bloodstream form strain 427 from K. Stuart, Seattle Biomedical Research Institute, Seattle, WA) was cultured in HMI-9 medium containing 10% fetal bovine serum, penicillin, and streptomycin at 37 °C with 5% CO_2.[48] Drug sensitivity of the *T. brucei* strain was determined in 96-well microtiter plates in triplicate with an initial inoculum of 5×10^3 trypomastigotes per well. Drugs were added in serial dilutions for a final volume of 200 μL/well. Parasite growth was quantified at 48 h by the addition of Alamar Blue (Alamar Biosciences, Sacramento, CA).[49] Alamar Blue quantification corresponded with microscopic counts determined with a hemacytometer. The Tulahuen strain of *T. cruzi* was provided by S. Reed (Infectious Diseases Research Institute, Seattle, WA). This strain was stably transfected with the *E. coli* β-galactosidase gene (lacZ) so that intracellular growth could be monitored with a colorimetric assay. The mammalian stages were grown in coculture with mouse 3T3 fibroblasts in RPMI 1640 containing 10% fetal bovine serum, penicllin, and streptomycin at 37 °C with 5% CO_2. Drug sensitivity studies with mammalian stage *T. cruzi* were carried out in triplicate using the β-galactosidase assay as described.[50]

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Supporting Information Available: Synthetic and analytical data for **1** and analytical data for N^6-(substituted)-2′-deoxy-2′-aminoadenosine and N^6-(substituted)-2′-deoxy-2′-(amido)adenosine analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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