Adenosine Analogues as Inhibitors of *Trypanosoma brucei* Phosphoglycerate Kinase: Elucidation of a Novel Binding Mode for a 2-Amino-N^6-Substituted Adenosine

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As part of a project aimed at structure-based design of adenosine analogues as drugs against African trypanosomiasis, N^6-, 2-amino-N^6-, and N^2-substituted adenosine analogues were synthesized and tested to establish structure−activity relationships for inhibiting *Trypanosoma brucei* glycosomal phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and glycerol-3-phosphate dehydrogenase (GPDH). Evaluation of X-ray structures of parasite PGK, GAPDH, and GPDH complexed with their adenosyl-bearing substrates led us to generate a series of adenosine analogues which would target all three enzymes simultaneously. There was a modest preference by PGK for N^6-substituted analogues bearing the 2-amino group. The best compound in this series, 2-amino-*N*^{6}-[2″-(*p*-hydroxyphenyl)ethyl]-adenosine (**46b**), displayed a 23-fold improvement over adenosine with an IC_{50} of 130 μM. 2-[[2″-(*p*-Hydroxyphenyl)ethyl]amino]adenosine (**46c**) was a weak inhibitor of *T. brucei* PGK with an IC_{50} of 500 μM. To explore the potential of an additive effect that having the N^6 and N^2 substitutions in one molecule might provide, the best ligands from the two series were incorporated into N^6,N^2-disubstituted adenosine analogues to yield *N*^{6}-(2″-phenylethyl)-2-[(2″-phenylethyl)amino]adenosine (**69**) as a 30 μM inhibitor of *T. brucei* PGK which is 100-fold more potent than the adenosine template. In contrast, these series gave no compounds that inhibited parasitic GAPDH or GPDH more than 10−20% when tested at 1.0 mM. A 3.0 Å X-ray structure of a *T. brucei* PGK/**46b** complex revealed a binding mode in which the nucleoside analogue was flipped and the ribosyl moiety adopted a syn conformation as compared with the previously determined binding mode of ADP. Molecular docking experiments using QXP and SAS program suites reproduced this "flipped and rotated" binding mode.

Introduction

African trypanosomiasis is a devastating parasitic disease that affects more than 300 000 people of sub-Saharan Africa each year.[1] The causative agent of this affliction is the protozoan *Trypanosoma brucei*. Upon taking a blood meal, *T. brucei* is introduced to the mammalian host via the salivary glands of the tsetse fly vector. *T. brucei* flourishes in the mammalian bloodstream, evading the immune response via antigenic variation, until it eventually finds its way across the blood−brain barrier and attacks the central nervous system.[2] This parasitic assault leads to dementia, epileptic attacks, coma, and eventually, if left untreated, death. Prevalent chemotherapeutic agents include Suramin, Pentamidine, Melarsoprol, and difluoromethylornithine (DFMO). All of these drugs have drawbacks such as limitations in use to the period before central nervous system involvement or to only one subspecies of the parasite, resistance, toxicity, or parenteral administration.[3,4]

In an effort to develop safer and more efficacious therapeutic agents for the treatment of African trypanosomiasis, we are focusing our efforts on trypanosome glycolysis as a target for structure-based drug design. It has rigorously been shown that upon going from insect to bloodstream form, *T. brucei* becomes solely dependent on glycolysis to the stage of pyruvate for its energy production.[5−7] As has been demonstrated by Clarkson and Brohn, disrupting carbohydrate catabolism in bloodstream form *T. brucei* significantly stifles parasite proliferation.[8] In computro modeling of parasite glycolytic flux suggests that, unlike in the mammalian pathway, glycosomal phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and glycerol-3-phosphate dehydrogenase (GPDH) all exert a significant amount of control over flux. At $[I]/K_I = 10-100$, competitive inhibitors of these enzymes may reduce glycolytic flux to zero.[9−11] Because of this unique influence on metabolic flux, it is surmised that inhibition of these particular enzymes may add a degree of selectivity for disrupting the parasite pathway over the mammalian one. Further selectivity will be sought by the design of compounds with greater affinity for the parasite over the human enzyme.

There is a wealth of crystallographic and sequence data on parasite and mammalian PGK,[12−15] GAPDH,[16−19]

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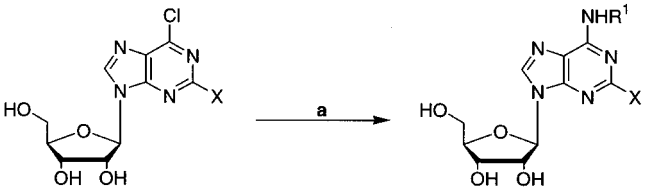
Scheme 1[a]



[a] Conditions: (a) R^1NH_2 and Et_3N in EtOH at 60 °C.

and GPDH[20] to use for structure-based design of competitive and parasite selective inhibitors. The majority of these structures contain the adenosyl-bearing cofactor bound in the active site, and thus indirectly we know the binding mode of the adenosyl moiety in all three of our targets. With this information, we chose to use adenosine as our template for inhibitor design. Work in our group has already had some success with generating N^6,2′-disubstituted adenosine analogues as inhibitors of parasitic GAPDH,[21,22] and it seemed reasonable to pursue a template which could be applied to all three targets simultaneously. Additionally, all three enzymes have common areas from which modifications to adenosine could extend. For example, PGK, GAPDH, and GPDH all have regions adjacent to the N^6, C2′, and C5′ positions of the adenosyl moiety that could accommodate modifications to the template.

We present a library of monosubstituted N^6 and N^2 adenosine analogues generated via solution-phase parallel synthesis. The binding affinity of these compounds for parasite PGK, GAPDH, and GPDH was determined, and the data led us to generate a small set of N^6,N^2-disubstituted adenosine analogues targeted at *T. brucei* PGK. It was clear, from analysis of the canonical binding mode of ADP to the kinase, that our most potent PGK inhibitors could not possibly adopt a binding mode similar to the adenosyl template of the ADP substrate. However, we obtained a 3.0 Å X-ray structure of a PGK/2-amino-N^6-substituted adenosine analogue complex that revealed a novel binding mode. This structure was used to test a number of molecular docking programs to see if they could reproduce the binding mode that was observed experimentally.

Results and Discussion

Chemistry. N^6-Substituted adenosines **2a**−**59a** and 2-amino-N^6-substituted adenosines **2b**−**59b** were synthesized via solution-phase parallel synthesis in one-step conversion from the commercially available 6-chloropurine riboside (**1a′**) and 2-amino-6-chloropurine riboside (**1b′**), respectively, by nucleophilic aromatic substitution with the appropriate amine at 60 °C (Scheme 1).[23,24] In a similar fashion, N^2-substituted adenosines **2c**−**59c** were prepared from 2-iodoadenosine (**1c′**) at 125 °C (Scheme 2).[25] Alternatively, compounds **2c**−**59c** could be synthesized as described by Francis et al. by using the commercially available 2-chloroadenosine.[26] We preferred not to use this route as we found it a bit more cost-effective to use **1c′**.

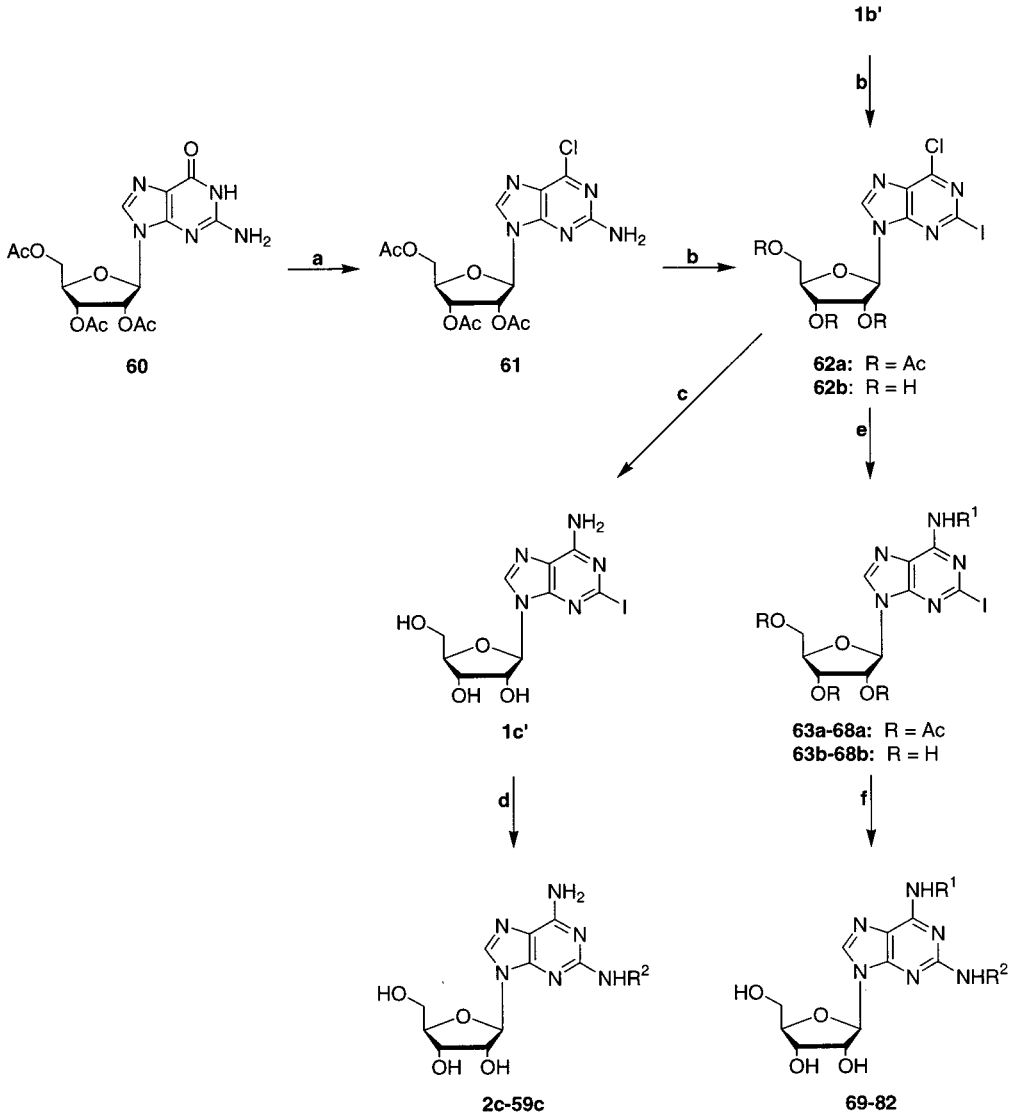
Previous attempts at synthesizing N^6,N^2-disubstituted adenosines by Trivedi et al. resulted in poor regioselectivity of the initial amination of 6-chloro-2-fluoropurine 9-(2′,3′,5′-triacetyl)riboside or involved C2 bromination of 2′,3′,5′-triacetylguanosine (**60**) followed by C2 amination, C6 chlorination, and C6 amination with subsequent deprotection of the hydroxyls.[27] We found that N^6,N^2-disubstituted adenosines could be readily synthesized from either 6-chloro-2-iodopurine 9-(2′,3′,5′-tri-*O*-acetyl)riboside (**62a**) or 6-chloro-2-iodopurine 9-riboside (**62b**) by taking advantage of the well-documented nucleophilic lability of the chloride of **62a** in the synthesis of **1c′** (Scheme 2).[28] Treatment of either **62a** or **62b** with 1.1 equiv of the amine to be substituted at the 6-position, a cobase, and heating at 60 °C afforded intermediates **63a**−**68a** or **63b**−**68b**, respectively, while in situ treatment of these compounds with a 20-fold excess of the amine to be substituted at the 2-position and heating at 125 °C yielded the desired disubstituted products **69**−**82**. Amination of **62a** invariably led to some degree of deacetylation of the desired intermediates **63a**−**68a** as determined by electrospray ionization mass spectrometry (ESI-MS). Amination of **62b** led to a relatively clean intermediate for which subsequent amination of **63b**−**68b** gave the desired products in 10−20% greater yields than those obtained with **62a** as the starting material.

Intermediate **62a** was synthesized as described by Matsuda et al. starting from commercially available 2′,3′,5′-triacetylguanosine (**60**).[29] We have shown that direct diazotization−iodination of **1b′** gives the intermediate 6-chloro-2-iodopurine 9-riboside (**62b**). The yield is not as robust as that obtained in the route starting with **60**, but this shorter synthetic route to the desired N^6,N^2-disubstituted compounds avoids the use of toxic and corrosive $POCl_3$. Ammoniaolysis of both **62a**[29] and **62b** affords **1c′** (Scheme 2).

Compounds **16a**−**20a** and **16b**−**20b** could also be synthesized by employing diaminoalkyltrityl resins. Coupling of **1a′** or **1b′** to these resins followed by cleavage of the alkylamino group with 30% hexafluoro-2-propanol (HFIP) in CH_2Cl_2[30] yielded the desired nucleosides in greater than 95% purity as determined by HPLC and in greater than 90% yield (Scheme 3). This route avoids the need for multiple crystallizations[31] or HPLC purification of these polar compounds when synthesized via Scheme 1. Alkyl-bridged bisadenosines (**16a′**−**20a′** and **16b′**−**20b′**) were either synthesized as described by Agathocleous et al.[32] or isolated as impurities generated from the synthesis of compounds **16a**−**20a** and **16b**−**20b** via Scheme 1.

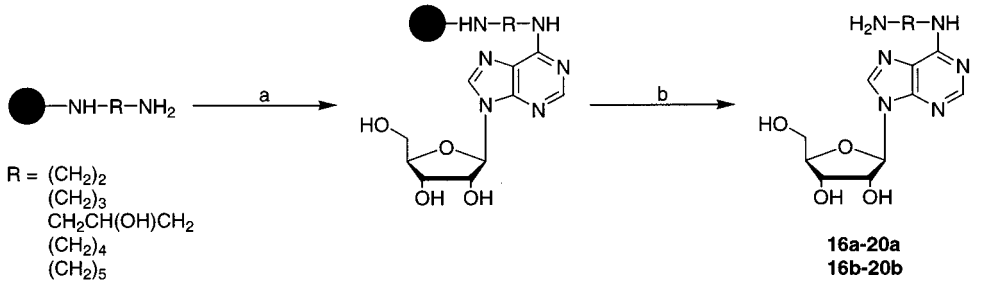
Structure−Activity Relationship Series. *T. brucei* PGK, GAPDH, and GPDH have regions near the N^6 position of the adenine ring of their bound adenosyl containing substrates for which modifications to the adenosine template could extend. For PGK, this small hydrophilic area is filled with three conserved water molecules. The large hydrophobic N^6 region of GAPDH has been previously described.[21,22] The N^6 region of GPDH points toward the protein surface/solvent interface and appears to be able to accommodate either hydrophobic or hydrophilic substituents depending on their orientation. The C6 amino group of the adenine ring makes critical hydrogen bonds in both the PGK and GAPDH structures, while it interacts with solvent in the GPDH structure. Because of our success with N^6

Scheme 2[a]



[a] Conditions: (a) *N*,*N*-dimethylaniline, $POCl_3$, Et_4NCl in ACN at 100 °C; (b) CuI, CH_2I_2, I_2 and isoamyl nitrite in THF at 80 °C; (c) NH_3 in MeOH at 60 °C; (d/f) R^2NH_2 in $MeOCH_2CH_2OH$ at 125 °C; (e) R^1NH_2 and Et_3N in $MeOCH_2CH_2OH$ at 60 °C.

Scheme 3[a]

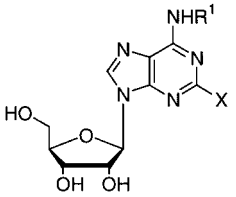


[a] Conditions: (a) trityl chloride resins, **1a** or **1b** in $EtOH/CH_2Cl_2$ (1:1) at 60 °C; (b) 30% $CF_3CH(OH)CF_3$ in CH_2Cl_2 at rt.

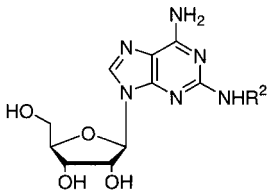
substituents for GAPDH and our desire to maintain the hydrogen bonds in the PGK and GAPDH structures, we chose to explore the attachment of substituents to the N^6 position. We attempted to pick a diverse group of ligands for the structure−activity relationship series (SAR) that would simultaneously accommodate all three enzymes and, at the same time, incorporate materials that were readily available commercially or from previous synthetic projects. Unfortunately, the screening of our small SAR library elucidated no inhibition greater than 10−20% against parasite GAPDH or GPDH, when tested at 1.0 mM, with the exception of **24a**, **29a**, **30a**, and **41a** for which potency against GAPDH was previously determined.[21]

With respect to PGK, these compounds were also fairly weak inhibitors. However, there was a general trend for 2-amino-N^6-substituted analogues to be more active than the corresponding N^6 analogues, which lacked the C2 amino group (Table 1). Our initial SAR led to the surprising result of 2-amino-N^6-(2″-phenyl-

Table 1. Inhibition of *T. brucei* PGK by N^6- and 2-Amino-N^6-Substituted Adenosine Analogues



1a-43a: X = H
1b-43b: X = NH_2



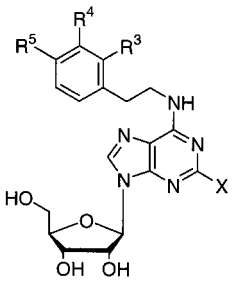
1c-43c

compd	R^1 or R^2	IC_{50} (mM) a	b	c
1	−H	3.0	3.5	
2	$-CH_3$	inactive[a]	inactive	NS[b]
3	$-CH_2CH_3$	inactive	3.5	NS
4	$-CH_2CH_2CH_3$	inactive	3.0	1.5
5	$-CH(CH_3)_2$	inactive	inactive	NS
6	$-CH_2(CH_2)_2CH_3$	inactive	0.9	1.5
7	$-CH_2(CH_2)_3CH_3$	3.0	2.0	inactive
8	$-CH_2CHCH_2$	inactive	3.0	NS
9	$-CH_2CCH$	inactive	1.5	NS
10	$-CH_2CH_2OH$	inactive	inactive	inactive
11	$-CH_2(CH_2)_2OH$	inactive	inactive	inactive
12	$-CH_2CH(OH)CH_3$	inactive	inactive	inactive
13	$-CH_2CH(OH)CH_2OH$	inactive	inactive	inactive
14	$-CH_2(CH_2)_3OH$	inactive	inactive	inactive
15	$-CH_2(CH_2)_4OH$	inactive	inactive	inactive
16	$-CH_2CH_2NH_2$	2.5	inactive	inactive
17	$-CH_2(CH_2)_2NH_2$	inactive	4.0	inactive
18	$-CH_2CH(OH)CH_2NH_2$	inactive	inactive	inactive
19	$-CH_2(CH_2)_3NH_2$	inactive	inactive	inactive
20	$-CH_2(CH_2)_4NH_2$	inactive	inactive	inactive
21	−cyclopentyl	3.9	1.5	1.5
22	−cyclohexyl	inactive	inactive	1.5
23	$-CH_2$-cyclohexyl	inactive	2.5	inactive
24	−cycloheptyl	3.9	2.5	3.0
25	−tetrahydrofurfuryl	inactive	inactive	0.8
26	−furfuryl	inactive	1.5	1.2
27	$-CH_2C_6H_5$	1.0	0.6	3.5
28	$-CH(CH_3)C_6H_5$	inactive	2.9	ns
29	$-CH_2$-(*o*-CH_3-C_6H_4)	inactive	inactive	1.5
30	$-CH_2$-(*m*-CH_3-C_6H_4)	inactive	0.4	1.5
31	$-CH_2$-(*p*-CH_3-C_6H_4)	inactive	inactive	1.5
32	$-CH_2$-(*o*-Cl-C_6H_4)	inactive	inactive	2.0
33	$-CH_2$-(*m*-Cl-C_6H_4)	inactive	inactive	0.8
34	$-CH_2$-(*p*-Cl-C_6H_4)	inactive	inactive	1.4
35	$-CH_2$-(*o*-OCH_3-C_6H_4)	inactive	inactive	1.4
36	$-CH_2$-(*p*-OCH_3-C_6H_4)	inactive	inactive	1.0
37	$-CH_2CH_2C_6H_5$	inactive	0.2	0.7
38	$-CH_2CH(CH_3)C_6H_5$	inactive	2.9	3.5
39	$-CH_2CH(OH)C_6H_5$	inactive	inactive	1.4
40	$-CH_2CH_2CH_2C_6H_5$	inactive	4.0	1.8
41	$-CH_2$-1-naphthalene	inactive	1.9	NS
42	$-CH(C_6H_5)CH_2(C_6H_5)$	inactive	inactive	NS
43	$-CH_2CH(C_6H_5)_2$	inactive	inactive	NS

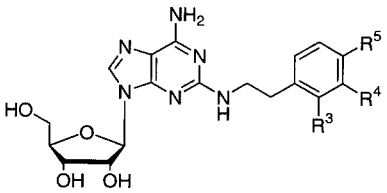
[a] Inactive = inactive at 4 mM. [b] NS = not synthesized.

ethyl)adenosine (**37b**) inhibiting *T. brucei* PGK with an IC_{50} of 200 μM. It was quite clear from analysis of the PGK/ADP structure[13] that the phenethyl substituent, along with many of the other N^6 ligands which showed activity, was not only too large to fit into the small pocket positioned adjacent to the N^6 region but also not well-suited to the hydrophilic nature of the cavity. Still, we pursued a small SAR subset of phenethyl analogues and found 2-amino-N^6-[2″-(*p*-hydroxyphenyl)ethyl]adenosine (**46b**) to be marginally better than the parent analogue, **37b**, with an IC_{50} of 130 μM (Table 2). This was a 23-fold improvement over the adenosine template which has an IC_{50} of 3000 μM. Adenosine (**1a**), 2-aminoadenosine (**1b**), **37b**, and **46b** were competitive with ATP and noncompetitive with 3-PGA (data not shown).

Table 2. Inhibition of *T. brucei* PGK by N^6-Phenethyl-, 2-Amino-N^6-phenethyl-, and N^2-Phenethyladenosine Analogues



44a-59a: X = H
44b-59b: X = NH_2



44c-59c

compd	R^3	R^4	R^5	IC_{50} (mM) a	b	c
37	H	H	H	inactive[a]	0.2	0.7
44	H	H	CH_3	inactive	2.0	inactive
45	H	OH	H	inactive	0.25	NS[b]
46	H	H	OH	inactive	0.13	0.5
47	H	OH	OH	inactive	0.25	NS
48	Cl	H	H	inactive	1.9	2.2
49	H	Cl	H	inactive	1.2	inactive
50	H	H	Cl	inactive	1.4	inactive
51	Cl	H	Cl	inactive	inactive	0.6
52	OCH_3	H	H	inactive	1.8	inactive
53	H	OCH_3	H	inactive	1.9	inactive
54	H	H	OCH_3	inactive	1.5	inactive
55	H	OCH_3	OCH_3	inactive	inactive	inactive
56	H	OCH_3	OH	inactive	1.5	NS
57	H	OH	OCH_3	inactive	0.85	NS
58	H	H	NH_2	inactive	0.25	0.5
59	H	H	NO_2	inactive	inactive	NS

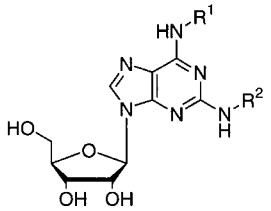
[a] Inactive = inactive at 4 mM. [b] NS = not synthesized.

Both **37b** and **46b** were 10-fold more selective for parasite PGK over rabbit muscle PGK (mammalian control).

The preference, by PGK, for the C2 NH_2 led us to explore N^2 adenosine analogues. Inspection of the available *T. brucei* PGK binary and ternary structures suggested that substitutions in this area would be well-tolerated by PGK. GPDH also has room to accommodate substituents in this position; however, GAPDH does not due to the snug fit of the adenosyl moiety in this region. Despite the modeling suggestions for GAPDH, the N^2 SAR was screened against all three target enzymes. None of the analogues inhibited *T. brucei* GAPDH or GPDH more than 10−20% at 1.0 mM, and a weak inhibitor, 2-[(2″-phenylethyl)amino]adenosine (**37c**), with an IC_{50} of 700 μM was found for PGK (Table 1). Again, we pursued a small SAR subset of N^2-phenethyl analogues and found 2-[[2″-(*p*-hydroxyphenyl)ethyl]amino]-adenosine (**46c**) to be marginally better than the parent analogue with an IC_{50} of 500 μM (Table 2). Both **37c** and **46c** were competitive with ATP, noncompetitive with 3-PGA (data not shown), and 4-fold more selective for parasite PGK over rabbit muscle PGK.

Attachment of the phenethyl groups on the adenine ring to give N^6,N^2-disubstituted adenosine analogues, targeted toward *T. brucei* PGK, yielded N^6-(2″-phenylethyl)-2-[(2″-phenylethyl)amino]adenosine (**69**) with an IC_{50} of 30 μM (100-fold more potent than adenosine). The addition of a *p*-hydroxyl group to just one of either of the phenethyl substituents, N^6-[2″-(*p*-hydroxyphenyl)-ethyl]-2-[(2″-phenylethyl)amino]adenosine (**72**) and N^6-(2″-phenylethyl)-2-[[2″-(*p*-hydroxyphenyl)ethyl]amino]-adenosine (**73**), decreased activity approximately 3-fold,

Table 3. Inhibition of *T. brucei* PGK by N^6,N^2-Diphenethyladenosine Analogues



69-82

compd	R^1	R^2	IC_{50} (μM)
69	$-CH_2CH_2C_6H_5$	$-CH_2CH_2C_6H_5$	30
70	$-CH_2CH_2$-(*p*-OH-C_6H_4)	$-CH_2CH_2$-(*p*-OH-C_6H_4)	inactive[a]
71	$-CH_2CH_2$-(*p*-NH_2-C_6H_4)	$-CH_2CH_2$-(*p*-NH_2-C_6H_4)	inactive
72	$-CH_2CH_2$-(*p*-OH-C_6H_4)	$-CH_2CH_2C_6H_5$	100
73	$-CH_2CH_2C_6H_5$	$-CH_2CH_2$-(*p*-OH-C_6H_4)	100
74	$-CH_2(CH_2)_2CH_3$	$-CH_2(CH_2)_2OH$	inactive
75	$-CH_2(CH_2)_2CH_3$	$-CH_2$-cyclohexyl	inactive
76	$-CH_2(CH_2)_2CH_3$	$-CH_2C_6H_5$	inactive
77	−tetrahydrofurfuryl	$-CH_2(CH_2)_2OH$	inactive
78	−tetrahydrofurfuryl	$-CH_2$-cyclohexyl	inactive
79	−tetrahydrofurfuryl	$-CH_2C_6H_5$	inactive
80	$-CH_2$-1-naphthalene	$-CH_2(CH_2)_2OH$	inactive
81	$-CH_2$-1-naphthalene	$-CH_2$-cyclohexyl	inactive
82	$-CH_2$-1-naphthalene	$-CH_2C6H_5$	inactive

[a] Inactive = inactive at 100 μM.

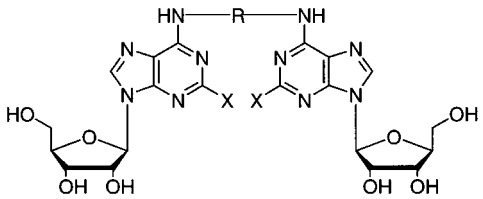
while the addition of a *p*-hydroxyl group to both of the phenethyl substituents, N^6-[2″-(*p*-hydroxyphenyl)ethyl]-2-[[2″-(*p*-hydroxyphenyl)ethyl]amino]adenosine (**70**), led to an inactive compound when tested at its solubility limit of 100 μM. Compound **69** was competitive with ATP, noncompetitive with 3-PGA (data not shown), and completely selective for parasite PGK over rabbit muscle PGK when tested up to its solubility limit of 100 μM.

As the substituents we chose to incorporate into our analogues were both similar and identical in structure (i.e. phenethyl), we generated a 3 × 3 matrix of randomly selected ligands and synthesized the respective compounds, **74**−**82**, to further demonstrate the utility of the chemistry. These compounds were inactive against *T. brucei* PGK when tested at 100 μM.

As an interesting aside, synthesis of compounds **16a**−**20a** and **16b**−**20b** via Scheme 1 invariably led to N^6,$N^{6\prime}$-alkanediylbisadenosines (**16a′**−**20a′** and **16b′**−**20b′**) for which **16a′**, **17a′**, **19a′**, and **20a′** are known inhibitors of adenosine kinase.[33] We isolated/synthesized the bisadenosines and screened them against parasite PGK to find that most of the C2 amino-bearing compounds had some marginal activity and N^6,$N^{6\prime}$-1,3-propanediyl-bis-2-aminoadenosine (**17b′**) had an IC_{50} of 50 μM (Table 4). Compound **17b′** was competitive with ATP, noncompetitive with 3-PGA (data not shown), and not selective for *T. brucei* PGK over rabbit muscle PGK.

Structure of the *T. brucei* PGK/2-Amino-N^6-[2″-(*p*-hydroxyphenyl)ethyl]adenosine (46b) Complex. Attempts at obtaining X-ray crystal structures of *T. brucei* PGK complexed with **46c** or **69** were unsuccessful; however, we were able to obtain an X-ray crystal structure of a *T. brucei* PGK/2-amino-N^6-[2″-(*p*-hydroxyphenyl)ethyl]adenosine (**46b**) complex (Figures 1 and 2). Quite remarkably, compound **46b** sits in the C-terminal ADP binding region such that the purine ring is flipped 180°, compared to bound ADP, along an axis that is approximately defined by N1 and N9 of the

Table 4. Inhibition of *T. brucei* PGK by *N*,*N*′-Alkanediylbisadenosine Analogues



16a′-20a′: X = H
16b′-20b′: X = NH_2

		IC_{50} (μM)	
compd	R	**a′**	**b′**
16	$-CH_2CH_2-$	inactive[a]	500
17	$-CH_2CH_2CH_2-$	inactive	50
18	$-CH_2CH(OH)CH_2-$	inactive	inactive
19	$-CH_2(CH_2)_2CH_2-$	inactive	750
20	$-CH_2(CH_2)_3CH_2-$	inactive	1000

[a] Inactive = inactive at 1000 μM.

adenine ring. The adenosine moiety of **46b** is also translated by approximately 1.5 Å in the adenine plane along the C6/N1 vector of the ADP substrate. In addition, the ribosyl moiety is rotated by 180° along the N9 and C1′ bond such that it now adopts a syn orientation to the adenine base rather than the anti orientation found for the bound ADP (Figure 3). Analysis of all nucleoside crystallographic data suggests that purine nucleosides adopt both syn and anti forms with a preference for the anti orientation.[34] Inspection of the 41 purine nucleoside analogues in the Cambridge database revealed that 14 out of 41 compounds adopt the syn conformation suggesting that the free energy difference between the two conformations is quite small.

The adenine ring of ADP, in *T. brucei* PGK, sits in a narrow hydrophobic cleft flanked by side chains Ala-218, Ala-242, and the methylenes of Lys-259 on one side and Val-343, Leu-315, and Phe-294 on the other (Figure 1). Adenine occupies part of the cleft leaving vacant space between Phe-294 and Lys-259. In the PGK/**46b** complex, the adenine base has been translated to sit between Phe-294 and Lys-259. In this "flipped and rotated" binding mode, the C2′ and C3′ hydroxyls of the ribose form hydrogen bonds with the same protein residue, Glu-345, as the respective hydroxyls of the ADP ribose. For the PGK/substrate complex, the C5′ oxygen does not participate in hydrogen bonding, and modeling suggests that, in the absence of phosphate, the proton of the hydroxyl would not be engaged in a hydrogen-bonding interaction with the protein. In contrast, as a result of the flipping and rotation of the sugar, the C5′ hydroxyl of **46b** participates in a hydrogen bond with the backbone amide of Ala-242.

The N^2 position of the inhibitor's adenine ring occupies approximately the N^6 of ADP (Figures 1 and 3), with the respective amino group in both molecules making a hydrogen bond with the backbone carbonyl of Ala-314. This, in part, explains the importance of the C2 amino group in the series of N^6-monosubstituted compounds. The conserved water molecules, observed in numerous PGK structures, were not observed in the structure of the **46b** complex due to the lower resolution of the latter structure. However, it is surmised that the

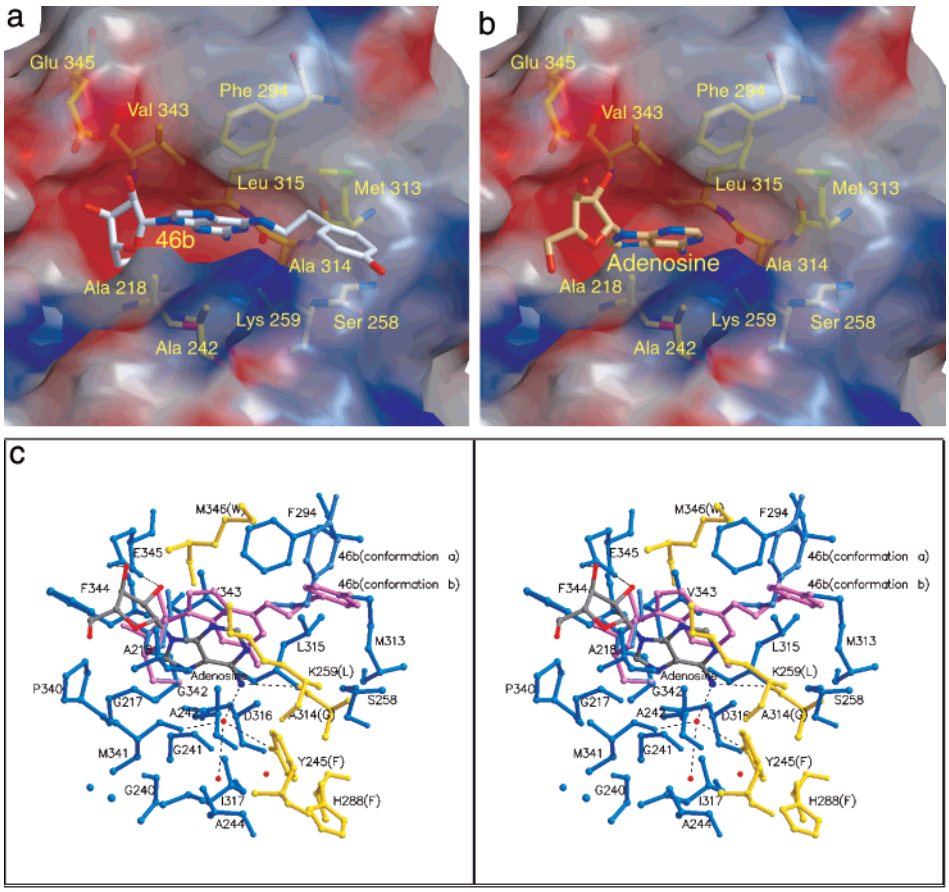


Figure 1. X-ray crystal structure of *T. brucei* PGK (a) complexed with compound **46b** and depicted with a molecular surface colored by electrostatic potential; (b) complexed with the adenosine moiety of ADP (from 2.5 Å structure) and depicted with a molecular surface colored by electrostatic potential; (c) with stereoview of the adenosine binding region shown with the two binding modes of **46b** (dark and light purple) and adenosine. Residues are colored blue if they are conserved between human and *T. brucei* enzymes and yellow if they are different (residues in parentheses correspond to human enzyme). Hydrogen bonds are drawn as dashed line. Generated using MOLSCRIPT,[49] GRASP,[50] and Raster3D.[51]

hydrogen bond that is observed in the ADP structure, between N^6 and one of the conserved waters, can also be made by the C2 amino group with minor rearrangement of a water molecule. In this binding mode, the C6 amino of **46b** is now directed almost precisely where the C2 proton of ADP is directed (Figure 3).

Both protein molecules in the asymmetric unit reveal bound inhibitor. Compound **46b** exhibits stronger electron density in PGK subunit A than in subunit B, which may be accounted for by different crystal packing environments of the inhibitor (Figure 2). The density of the hydroxyphenethyl group of the inhibitor in molecule A has branches in two directions suggesting alternative binding modes. In one binding mode, the phenyl ring leans on the hydrophobic protein surface formed by Phe-294 and Met-313. In the second, the ring makes hydrophobic interactions with the methylene chain of Lys-259 and the backbone of Ser-258. In both cases, the N^6 proton and the hydroxyl of the phenethyl group are exposed to solvent and do not appear to contribute to hydrogen bonding with the protein. We considered synthesizing a molecule that could simultaneously take advantage of the two orientations of the phenethyl substituent; however, it was clear from modeling experiments that any compound that could maintain the distinct phenethyl orientations would suffer from severe intramolecular clashes between the phenyl rings.

Without a structure of the PGK/**46c** complex, it is difficult to comment on the binding mode of this compound that bears the same substituent as **46b**. Molecular modeling studies suggest that **46c** could indeed sit in the ADP active site in such a fashion as to have the adenosyl moieties superimposed and such that the *p*-hydroxyphenethyl substituent sits in the same region as that of **46b** in the PGK/**46b** complex. The modest selectivity experienced by **46b** for *T. brucei* PGK may be accounted for by Lys-259 being replaced by a leucine in the mammalian enzyme. Although this structure has provided insight into how the 2-amino-N^6-substituted adenosine analogue binds, it is unlikely that compound **69** could bind in a similar fashion as that of **46b** or ADP since the N^2-phenethyl group has merely exchanged places with the N^6-phenethyl group for which there is still not enough room to be accommodated.

Docking Experiments. Compound **46b** was docked by SAS[35] (Stochastic Approximation with Smoothing) and QXP[36] in an effort to see if these programs could reproduce the crystallographic binding mode. Although both programs successfully found the "flipped and rotated" binding mode of **46b** when a 1.6 Å PGK crystal structure[14] was used (data not shown), only the program SAS gave acceptable results when the 3.0 Å *T. brucei* PGK structure was used.

For the docking experiment using the SAS program, 50 runs of 1000 cycles were performed to dock **46b** to

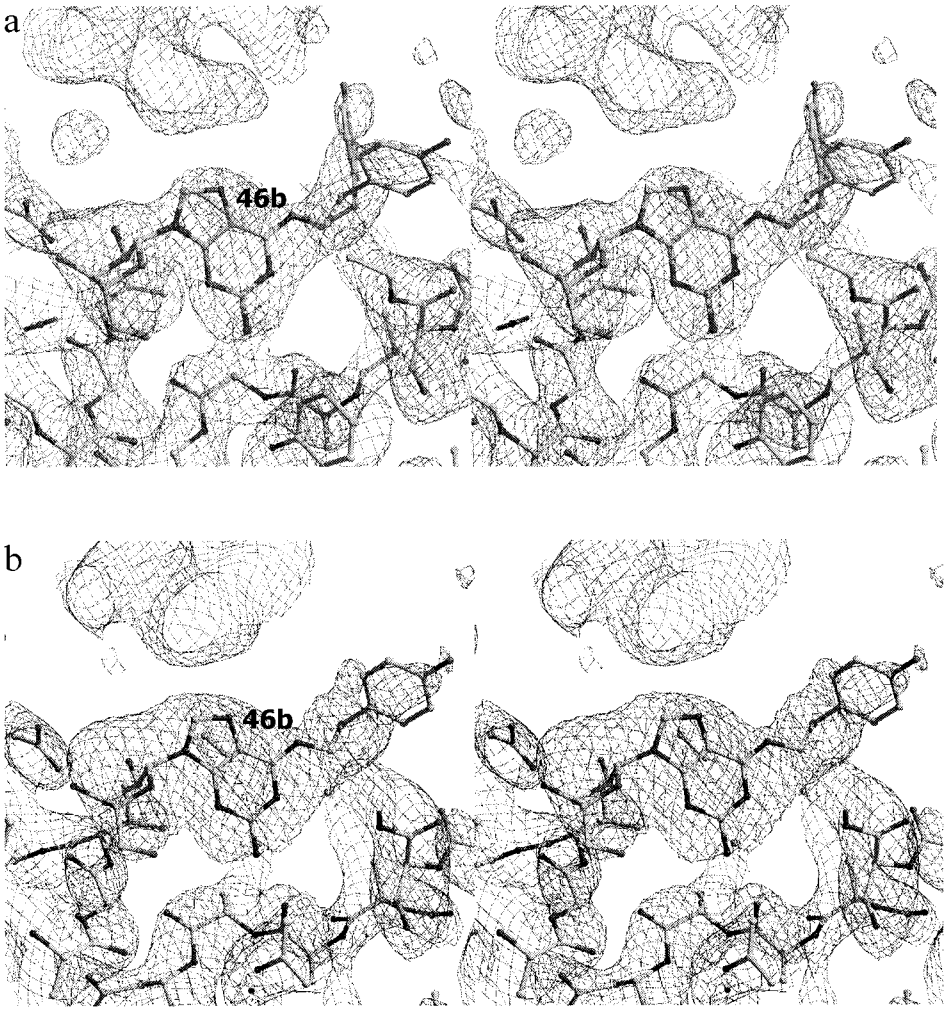


Figure 2. Stereoview of simulated annealing omit map ($2F_o - F_c$, 1.0σ) around **46b** in (a) subunit A with the two binding modes of the *p*-hydroxyphenethyl substituents and (b) subunit B. Prepared using the programs XtalView[52] and Raster3D.[51]

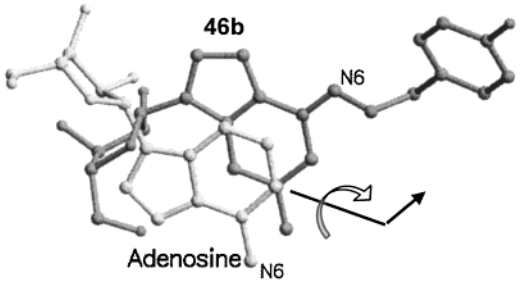


Figure 3. Superposition of the bound conformations of adenosine (light gray, from PGK ternary complex structure[13]) and **46b** (dark gray) in *T. brucei* PGK. N^6 positions of both molecules are marked for comparison. Arrows indicate the rotation and translation of the purine moiety.

the 3.0 Å *T. brucei* PGK structure, and results with rmsd's smaller than 1.0 Å were clustered (Table 5). The program found the flipped binding mode of adenosine in all top 10 ranks, and the sugar moiety was rotated with respect to the nucleoside base in all cases except ranks 5 and 6. The program also successfully positioned the *p*-hydroxyphenethyl substituent of **46b** in the two alternative binding modes observed crystallographically, and the results were clustered in two categories that represented the two observed orientations of the substituent: "A-like" and "B-like" (Figure 4); rmsd comparison of the adenosine moiety of **46b,** from the docking

Table 5. Statistics from Docking Experiments of **46b** into the 3.0 Å *T. brucei* PGK Structure with the Program SAS[35]

cluster rank	min/max score[a] (kcal/mol)	size of cluster[b]	rmsd[c] (Å) compared to conf A	rmsd[c] (Å) compared to conf B	rmsd[c] (Å) adensoine moiety only
1	−61.9/−58.9	8	1.64		0.56
2	−61.4/−56.8	6		1.72	0.61
3	−60.7/−59.5	2		1.87	0.64
4	−60.7/−57.5	13	1.78		1.25
5	−60.5/−60.4	2	1.94		2.67
6	−60.2/−60.2	1	2.06		2.95
7	−59.8/−59.8	1		1.53	0.59
8	−59.7/−58.0	8		2.25	1.38
9	−59.2/−59.2	1	1.70		2.73
10	−58.6/−57.7	2	1.52		1.18

[a] Min/max score = minimum and maximum energy score (kcal/mol) of the docked structures in the cluster. [b] Size of cluster = number of docked structures with rmsd's within 1.0 Å of each other. [c] rmsd = rmsd's (Å) of docked **46b** compared with conformation A of **46b**, conformation B of **46b**, and with only the adenosine moiety of the crystallographically determined orientation of **46b**.

experiment, showed that, in most cases, this portion of the inhibitor had a smaller rmsd compared with that of the complete **46b** molecule from the PGK/**46b** structure. In the three top-ranked clusters (Table 5), the agreement between the docked and the experimental binding modes only varied between 0.50 and 0.64 Å.

Trypanosomatid Growth Inhibition Studies. Our best inhibitor, **69**, was tested for the ability to block the growth of two strains of bloodstream *T. brucei*. The

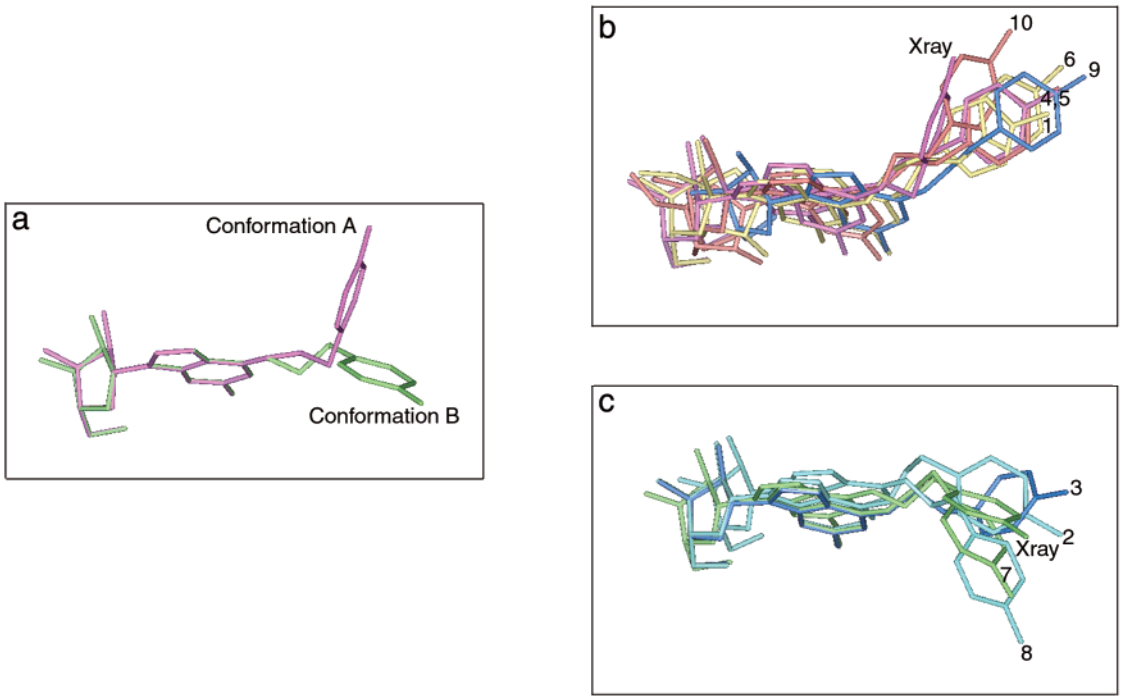


Figure 4. Docking results of **46b** to the PGK crystal structure using the program SAS: (a) binding modes of **46b** observed in the crystal structure; (b) superposition of conformation A with docking results (clusters 1, 4−6, 9, and 10, Table 5) that have "A-like" conformations; (c) superposition of conformation B with docking results (clusters 2, 3, 7, and 8, Table 5) that have "B-like" conformations.

Table 6. Data Collection and Refinement Statistics for the *T. brucei* PGK/**46b** Complex

Data Collection	
resolution (Å)	3.0
space group	$P6_322$ ($a = b = 186.6$ Å, $c = 159.0$ Å)
number of unique reflections	32896 (617652)[a]
completeness (%)	98.9 (99.5)[b]
$I/\sigma(I)$	28.0 (5.6)[b]
R_{merge} (%)	7.0 (41.8)[b]
Refinement	
number of protein atoms	6254
number of ligand atoms	58
resolution (Å)	15−3.0
σ cut-off	$F > 2\sigma(F)$
number of reflections	31864
R_{cryst} (%)	21.7
R_{free} (%)	28.2[c]
rmsd from ideality:	
bond length (Å)	0.020
angle (deg)	1.89
average *B* factor:	
main chain (Å^2)	29.7 (subunit A), 33.7 (subunit B)
side chain (Å^2)	36.4 (subunit A), 39.7 (subunit B)
ligand (Å^2)	35.0 (subunit A), 45.6 (subunit B)

[a] Number of total reflections. [b] Values in parentheses refer to data in the last shell between 3.3 and 3.0 Å resolution. [c] R_{free} was calculated for 5% of reflections omitted from the refinement.

concentration of inhibitor that caused 50% growth inhibition of both *T. brucei brucei* and *T. brucei rhodesiense* was 20 μM. We also tested the activity of this inhibitor against the trypanosome species that causes Chagas disease, *Trypanosoma cruzi*. The concentration of the inhibitor that caused 50% growth inhibition of mammalian stages of *T. cruzi* grown in murine fibroblasts was also 20 μM. The concentration of the inhibitor that inhibited 50% growth of the murine fibroblasts was 40 μM. All cell culture assays were performed twice with very similar results.

Conclusions

Utilizing structure-based design methodologies, we identified regions of our adenosine template for which modifications could be tolerated by our target enzymes. The quasi-rational selection of ligands to be incorporated into our molecules, based on diversity and availability, led to our most potent compound, **69**, which binds 100-fold tighter to PGK than our adenosine template. A crystallographic structure has revealed the detailed interactions between **46b** and *T. brucei* glycosomal PGK, including an entirely different binding mode than previously seen for ADP, and has also provided a structural rationale for the observed activities. This structure, used in conjunction with the SAS program which successfully reproduced the unexpected binding mode, has provided a direction for future attempts to improve binding affinity of this class of adenosine analogues.

Experimental Section

General Synthetic Procedures. ^{1}H NMR spectra were recorded on a Bruker AF-300 MHz or AM-500 MHz spectrometer in DMSO-d_6 unless otherwise stated. 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 $CHCl_3$/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, 100% 10 mM triethylammonium acetate (pH = 7.0) for 5 min then linear gradient of 10 mM triethylammonium acetate (pH = 7.0) from 0 to 15% over 60 min at 4.0 mL/min; method B, linear gradient of 5% HCOOH from 0 to 100% MeOH over 60 min at 4.0 mL/min; method C, linear gradient of 10 mM triethylammonium acetate (pH = 7.0) from 0 to 100% over 60 min at 4.0 mL/min. Purity of all compounds

was verified by HPLC with methods B and C on a C_{18} column (Vydac 218TP52) at 1.0 mL/min.

THF was freshly distilled over Na/benzophenone under argon, and CH_2Cl_2 was freshly distilled over CaH under argon. Adenosine (**1a**), 6-chloropurine riboside (**1a′**), 2-amino-6-chloropurine riboside (**1b′**), N^6-benzyladenosine (**27a**), and 2′,3′,5′-triacetylguanosine (**60**) were purchased from Aldrich Chemical Co. Ltd. N^6-Methyladenosine (**2a**) and N^6-(2″-hydroxyethyl)-adenosine (**10a**) were purchased from Sigma Chemical Co. 2-Aminoadenosine (**1b**) was purchased from R. I. Chemical. 3-Hydroxyphenethylamine hydrochloride, 3-hydroxy-4-methoxyphenethylamine hydrochloride, and 4-hydroxy-3-methoxyphenethylamine hydrochloride were purchased from Trans World Chemical Inc. Trityl chloride resin (swelling volume in CH_2Cl_2: 2−5 mL/g, loading: 0.95 mmol/g of active chloride, polymer matrix: copoly(styrene-2% DVB), 200−400 mesh) was purchased from Novabiochem. The diaminoalkyltrityl resins, which were prepared for these experiments using standard methods, can also be purchased through Novabiochem.

General Procedure for the Preparation of N^6- and 2-Amino-N^6-Substituted Adenosine Analogues (2a−59a and 2b−59b). In parallel fashion and with minor modifications, the method of Fleysher was employed.[23,24] To a suspension of **1a′** or **1b′** (0.017 mmol) in EtOH (250 μL) were added the appropriate amine or amine hydrochloride (0.05 mmol) and then triethylamine (0.05 mmol for free amines or 0.10 mmol for amine hydrochlorides). The reactions were heated at 60 °C for 18 h and then cooled to ambient temperature. The reaction mixtures were purified via flash chromatography, except for compounds **16a**−**20a** and **16b**−**20b** which were purified via preparative HPLC using method A, to yield the desired products in 75−95% yield.

2-Amino-N^6-isopropyladenosine (5b): ^{1}H NMR δ 1.16 (d, 6H, C2″-H), 3.52 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.07 (dd, 1H, C3′-H), 4.41 (br s, 1H, C1″-H), 4.55 (dd, 1H, C2′-H), 5.10 (d, 1H, OH), 5.35 (d, 1H, OH), 5.47 (br s, 1H, OH), 5.70 (d, 1H, C1′-H), 5.74 (br s, 2H, C2-NH_2), 7.05 (br s, 1H, N^6-H), 7.89 (s, 1H, C8−H); ESI-MS m/z 325.3 $(M + H^+)^+$.

2-Amino-N^6-(*n*-amyl)adenosine (7b): ^{1}H NMR (CD_3OD) δ 0.92 (t, 3H, C5″-H), 1.37 (m, 4H, C3″-H and C4”-H), 1.64 (m, 2H, C2″-H), 3.48 (br s, 2H, C1″-H), 3.75 and 3.87 (2 × dd, 2H, C5′-$H_{a,b}$), 4.14 (m, 1H, C4′-H), 4.28 (dd, 1H, C3′-H), 4.74 (dd, 1H, C2′-H), 5.77 (d, 1H, C1′-H), 7.83 (s, 1H, C8-H); ESI-MS m/z 353.2 $(M + H^+)^+$.

2-Amino-N^6-allyladenosine (8b): ^{1}H NMR (CD_3OD) δ 3.74 and 3.87 (2 × dd, 2H, C5′-$H_{a,b}$), 4.14 (m, 3H, C4′-H and C1″-H), 4.29 (dd, 1H, C3′-H), 4.74 (dd, 1H, C2′-H), 5.12 and 5.24 (2 × dd, 2H, C3″-H), 5.78 (d, 1H, C1′-H), 5.97 (m, 1H, C2″-H), 7.85 (s, 1H, C8-H); ESI-MS m/z 323.3 $(M + H^+)^+$.

***N*^{6}-Propargyladenosine (9a):** ^{1}H NMR δ 3.04 (br s, 1H, C3″-H), 3.55 and 3.66 (2 × m, 2H, C5′-$H_{a,b}$), 3.95 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.24 (br s, 2H, C1″-H), 4.59 (dd, 1H, C2′-H), 5.19 (d, 1H, OH), 5.35 (m, 1H, OH), 5.45 (d, 1H, OH), 5.89 (d, 1H, C1′-H), 8.25 (br s, 1H, N^6-*H*), 8.27 and 8.40 (2 × s, 2H, C2-H and C8-H); ESI-MS m/z 306.3 $(M + H^+)^+$.

2-Amino-N^6-propargyladenosine (9b): ^{1}H NMR (CD_3OD) δ 2.59 (br s, 1H, C3″-H), 3.72 and 3.87 (2 × dd, 2H, C5′-$H_{a,b}$), 4.14 (m, 1H, C4′-H), 4.28 (dd, 1H, C3′-H), 4.30 (br s, 2H, C1″-H), 4.75 (dd, 1H, C2′-H), 5.78 (d, 1H, C1′-H), 7.86 (s, 1H, C8-H); ESI-MS m/z 321.3 $(M + H^+)^+$.

2-Amino-N^6-(3″-hydroxypropyl)adenosine (11b): ^{1}H NMR δ 1.71 (p, 2H, C2″-H), 3.38 (m, 2H, C1″-H), 3.49 (m, 2H, C3″-H), 3.51 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.48 (dd, 1H, C2′-H), 4.58 (br s, 1H, C3″-O*H*), 5.14 (d, 1H, O*H*), 5.39 (d, 1H, O*H*), 5.45 (br s, 1H, O*H*), 5.71 (d, 1H, C1′-H), 5.83 (br s, 2H, C2-NH_2), 7.39 (br s, 1H, N^6-H), 7.93 (s, 1H, C8-H); ESI-MS m/z 341.2 $(M + H^+)^+$.

2-Amino-N^6-(2″-hydroxypropyl)adenosine (12b): ^{1}H NMR δ 1.05 (d, 3H, C3″-H), 3.31 and 3.40 (2 × m, 2H, C1″-$H_{a,b}$), 3.52 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.83 (m, 1H, C2″-H), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.47 (dd, 1H, C2′-H), 4.90 (br s, 1H, C2″-O*H*), 5.12 (d, 1H, O*H*), 5.38 (d, 1H, O*H*), 5.42 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 5.84 (br s, 2H, C2-NH_2), 7.04 (br s, 1H, N^6-H), 7.92 (s, 1H, C8-H); ESI-MS m/z 341.2 $(M + H^+)^+$.

2-Amino-N^6-(4″-hydroxybutyl)adenosine (14b): ^{1}H NMR δ 1.43 (p, 2H, C2”-H), 1.56 (p, 2H, C3″-H), 3.38 (m, 4H, C1″-H and C3″-H), 3.51 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.40 (br s, 1H, C3”-O*H*), 4.47 (dd, 1H, C2′-H), 5.12 (d, 1H, O*H*), 5.38 (d, 1H, O*H*), 5.46 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 5.83 (br s, 2H, C2-NH_2), 7.30 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS m/z 355.1 $(M + H^+)^+$.

***N*^{6}-(5″-Hydroxypentyl)adenosine (15a):** ^{1}H NMR δ 1.32 (p, 2H, C3″-H), 1.43 (p, 2H, C2″-H), 1.56 (p, 2H, C4″-H), 3.38 (m, 4H, C1″-H and C5″-H), 3.53 and 3.64 (2 × m, 2H, C5′-$H_{a,b}$), 3.95 (m, 1H, C4′-H), 4.12 (dd, 1H, C3′-H), 4.36 (t, 1H, C5″-OH), 4.60 (dd, 1H, C2′-H), 5.20 (d, 1H, O*H*), 5.44 (m, 2H, O*H*), 5.86 (d, 1H, C1′-H), 7.89 (br s, 1H, N^6-H), 8.19 and 8.33 (2 × s, 2H, C2-H and C8-H); ESI-MS m/z 354.2 $(M + H^+)^+$.

2-Amino-N^6-(5″-hydroxypentyl)adenosine (15b): ^{1}H NMR δ 1.30 (p, 2H, C3″-H), 1.43 (p, 2H, C2″-H), 1.56 (p, 2H, C4″-H), 3.37 (m, 4H, C1″-H and C5”-H), 3.50 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.36 (t, 1H, C5″-OH), 4.48 (dd, 1H, C2′-H), 5.14 (d, 1H, O*H*), 5.39 (d, 1H, O*H*), 5.45 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 5.83 (br s, 2H, C2-NH_2), 7.29 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS m/z 369.2 $(M + H^+)^+$.

2-Amino-N^6-(cyclohexylmethyl)adenosine (23b): ^{1}H NMR δ 0.80−1.80 (band, 11H, cyclohexyl-H), 3.22 (br s, 2H, N^6-CH_2), 3.52 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.90 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 5.10 (d, 1H, O*H*), 5.37 (d, 1H, O*H*), 5.43 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 5.74 (br s, 2H, C2-NH_2), 7.28 (br s, 1H, N^6-H), 7.89 (s, 1H, C8-H); ESI-MS m/z 379.2 $(M + H^+)^+$.

2-Amino-N^6-cycloheptyladenosine (24b): ^{1}H NMR (CD_3-OD) δ 1.53−2.01 (band, 12H, cycloheptyl-CH_2), 3.71 and 3.87 (2 × dd, 2H, C5′-$H_{a,b}$), 4.13 (m, 1H, C4′-H), 4.28 (m, 2H, C3′-H and C1”-H), 4.74 (dd, 1H, C2′-H), 5.76 (d, 1H, C1′-H), 7.83 (s, 1H, C8-H); ESI-MS m/z 379.3 $(M + H^+)^+$.

2-Amino-N^6-tetrahydrofurfuryladenosine (25b): ^{1}H NMR δ 1.59−1.88 (band, 4H, C3″- and C4″-H), 3.41−3.80 (band, 6H, N^6-CH_2, C5′-$H_{a,b}$, and C5″-H), 3.88 (m, 1H, C4′-H), 4.02 (m, 1H, C2″-H), 4.08 (dd, 1H, C3′-H), 4.47 (dd, 1H, C2′-H), 5.10 (d, 1H, O*H*), 5.36 (d, 1H, O*H*), 5.42 (br s, 1H, O*H*), 5.71 (d, 1H, C1′-H), 5.82 (br s, 2H, C2-NH_2), 7.14 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS m/z 367.2 $(M + H^+)^+$.

2-Amino-N^6-(*m*-methylbenzyl)adenosine (30b): ^{1}H NMR δ 2.26 (s, 3H, Ph-CH_3), 3.55 and 3.65 (2 × m, 2H, C5′-$H_{a,b}$), 3.90 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 4.62 (br s, 2H, N^6-CH_2), 5.09 (d, 1H, OH), 5.34 (d, 1H, OH), 5.41 (br s, 1H, OH), 5.72 (d, 1H, C1′-H), 5.79 (br s, 2H, C2-NH_2), 6.70 (d, 1H, Ph-H), 7.15 (m, 3H, Ph-H), 7.82 (br s, 1H, N^6-H), 7.92 (s, 1H, C8-H); ESI-MS m/z 387.2 $(M + H^+)^+$.

2-Amino-N^6-(*p*-methylbenzyl)adenosine (31b): ^{1}H NMR δ 2.27 (s, 3H, Ph-CH_3), 3.53 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.90 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.52 (dd, 1H, C2′-H), 4.64 (br s, 2H, N^6-CH_2), 5.09 (d, 1H, OH), 5.34 (d, 1H, OH), 5.41 (br s, 1H, OH), 5.72 (d, 1H, C1′-H), 5.79 (br s, 2H, C2-NH_2), 6.75 (d, 2H, Ph-H), 7.03 (d, 2H, Ph-H), 7.87 (br s, 1H, N^6-H), 7.98 (s, 1H, C8-H); ESI-MS m/z 387.2 $(M + H^+)^+$.

2-Amino-N^6-(*m*-chlorobenzyl)adenosine (33b): ^{1}H NMR δ 3.53 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 4.61 (br s, 2H, N^6-CH_2), 5.11 (br s, 1H, OH), 5.44 (br s, 2H, OH), 5.72 (d, 1H, C1′-H), 5.86 (br s, 2H, C2-NH_2), 7.21−7.39 (band, 4H, Ph-H), 7.94 (s, 1H, C8-H), 7.99 (br s, 1H, N^6-H); ESI-MS m/z 407.3 $(M + H^+)^+$.

2-Amino-N^6-(*p*-methoxybenzyl)adenosine (36b): ^{1}H NMR δ 3.53 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.70 (s, 3H, Ph-OCH_3), 3.90 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 4.55 (br s, 2H, N^6-CH_2), 5.11 (d, 1H, OH), 5.36 (d, 1H, OH), 5.41 (br s, 1H, OH), 5.71 (d, 1H, C1′-H), 5.82 (br s, 2H, C2-NH_2), 6.83 (d, 2H, Ph-H), 7.25 (d, 2H, Ph-H), 7.80 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS m/z 403.2 $(M + H^+)^+$.

2-Amino-N^6-(2″-hydroxy-2″-phenylethyl)adenosine (39b): ^{1}H NMR δ 3.55 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.77 (br s, 2H, C1″-H), 3.90 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.59 (dd, 1H, C2′-H), 4.85 (br s, 1H, C2″-H), 5.08 (d, 1H, O*H*), 5.33 (d, 1H, O*H*), 5.36 (m, 1H, O*H*), 5.62 (br s, 1H, C2″-O*H*), 5.72 (d, 1H, C1′-H), 5.87 (br s, 2H, C2-NH_2), 6.98 (br s, 2H, N^6-H),

7.23 (t, 1H, Ph-H), 7.32 (t, 1H, Ph-H), 7.41 (d, 1H, Ph-H), 7.92 (s, 1H, C8-H); ESI-MS *m/z* 403.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(3″-phenylpropyl)adenosine (40b): ^{1}H NMR (CD_3OD) δ 1.93 (p, 2H, 2″-H), 2.69 (t, 2H, 3″-H), 3.50 (br s, 2H, 1″-H), 3.71 and 3.86 (2 × m, 2H, C5′-$H_{a,b}$), 4.14 (m, 1H, C4′-H), 4.28 (dd, 1H, C3′-H), 4.75 (dd, 1H, C2′-H), 5.77 (d, 1H, C1′-H), 7.12−7.24 (band, 5H, Ph-H), 7.83 (s, 1H, C8-H); ESI-MS *m/z* 401.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(1″-naphthylmethyl)adenosine (41b): ^{1}H NMR δ 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 5.11 (m, 3H, O*H* and C1″-H), 5.38 (m, 2H, O*H*), 5.73 (d, 1H, C1′-H), 5.84 (br s, 2H, C2-NH_2), 7.41−8.23 (band, 9H, N^6-H, C8-H, and naphthyl-H); ESI-MS *m/z* 423.2 $(M + H^+)^+$.

***N*^{6}-(1″,2″-Diphenethyl)adenosine (42a):** ^{1}H NMR δ 3.08 and 3.32 (2 × m, 2H, C2″-H), 3.54 and 3.65 (2 × m, 2H, C5′-$H_{a,b}$), 3.95 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.59 (dd, 1H, C2′-H), 5.20 (br s, 1H, OH), 5.39 (br s, 1H, OH), 5.48 (br s, 1H, OH), 5.52 (m, 1H, C1″-H), 5.87 (d, 1H, C1′-H), 7.09−8.55 (band, 10H, Ph-H), 8.23 and 8.34 (2 × s, 1H, C2-H and C8-H), 8.44 (d, 1H, N^6-H); ESI-MS *m/z* 448.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(1″,2″-diphenethyl)adenosine (42b): ^{1}H NMR δ 3.08 and 3.32 (2 × m, 2H, C2″-H), 3.55 and 3.65 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.11 (br s, 1H, OH), 5.36 (br s, 1H, OH), 5.44 (br s, 1H, OH), 5.56 (m, 1H, C1″-H), 5.70 (d, 1H, C1′-H), 5.74 (br s, 2H, C2-NH_2), 7.09−8.55 (band, 10H, Ph-H), 7.91 (s, 1H, C8-H), 7.92 (d, 1H, N^6-H); ESI-MS *m/z* 463.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-methylphenyl)ethyl]adenosine (44b): ^{1}H NMR δ 2.25 (s, 3H, Ph-CH_3), 2.84 (t, 2H, C2″-H), 3.52 and 3.64 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.89 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.50 (dd, 1H, C2′-H), 5.07 (d, 1H, O*H*), 5.32 (m, 1H, O*H*), 5.40 (d, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.78 (br s, 2H, C2-NH_2), 7.07 (d, 2H, Ph-H), 7.14 (d, 2H, Ph-H), 7.25 (br s, 1H, N^6-H), 7.89 (br s, 1H, C8-H); ESI-MS *m/z* 401.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-hydroxyphenyl)ethyl]adenosine (46b): ^{1}H NMR δ 2.76 (t, 2H, C2″-H), 3.53 and 3.62 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.90 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.50 (dd, 1H, C2′-H), 5.10 (t, 1H, O*H*), 5.35 (d, 1H, O*H*), 5.48 (m, 1H, O*H*), 5.78 (d, 1H, C1′-H), 5.82 (br s, 2H, C2-NH_2), 6.65 (d, 2H, Ph-H), 7.04 (d, 2H, Ph-H), 7.41 (t, 1H, N^6-H), 7.92 (s, 1H, C8-H), 9.18 (s, 1H, Ph-O*H*); ESI-MS *m/z* 403.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*m*,*p*-dihydroxyphenyl)ethyl]adenosine (47b): ^{1}H NMR δ 2.68 (t, 2H, C2″-H), 3.39 (br s, 2H, C1″-H), 3.51 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.50 (dd, 1H, C2′-H), 5.20 (br s, 1H, O*H*), 5.42 (br s, 2H, O*H*), 5.77 (d, 1H, C1′-H), 5.83 (br s, 2H, C2-NH_2), 6.48 (d, 1H, Ph-H), 6.63 (m, 2H, Ph-H), 7.23 (br s, 1H, N^6-H), 7.93 (s, 1H, C8-H), 8.75 (br s, 2H, Ph-O*H*); ESI-MS *m/z* 419.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*o*-chlorophenyl)ethyl]adenosine (48b): ^{1}H NMR δ 3.07 (t, 2H, C2″-H), 3.55 and 3.65 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.94 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.54 (dd, 1H, C2′-H), 5.12 (d, 1H, O*H*), 5.38 (d, 1H, O*H*), 5.45 (br s, 1H, O*H*), 5.76 (d, 1H, C1′-H), 5.80 (br s, 2H, C2-NH_2), 7.25 (m, 2H, Ph-H), 7.45 (m, 3H, N^6-H and Ph-H), 7.94 (s, 1H, C8-H); ESI-MS *m/z* 421.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*m*-chlorophenyl)ethyl]adenosine (49b): ^{1}H NMR δ 2.90 (t, 2H, C2″-H), 3.55 and 3.65 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.48 (dd, 1H, C2′-H), 5.07 (d, 1H, O*H*), 5.33 (d, 1H, O*H*), 5.45 (br s, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.82 (br s, 2H, C2-NH_2), 7.23 (t, 1H, Ph-H), 7.30 (m, 3H, N^6-H and Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 421.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-chlorophenyl)ethyl]adenosine (50b): ^{1}H NMR δ 2.90 (t, 2H, C2″-H), 3.55 and 3.65 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.89 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.50 (dd, 1H, C2′-H), 5.08 (d, 1H, O*H*), 5.33 (d, 1H, O*H*), 5.45 (br s, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.81 (br s, 2H, C2-NH_2), 7.28 (d, 2H, Ph-H), 7.32 (d, 2H, Ph-H), 7.35 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS *m/z* 421.1 $(M + H^+)^+$.

***N*^{6}-[2″-(*o*,*p*-Dichlorophenyl)ethyl]adenosine (51a):** ^{1}H NMR δ 3.04 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 2H, C5′-$H_{a,b}$), 3.72 (m, 2H, C1″-H), 3.96 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.16 (d, 1H, O*H*), 5.36 (m, 1H, O*H*), 5.41 (d, 1H, O*H*), 5.88 (d, 1H, C1′-H), 7.32 (m, 2H, Ph-H), 7.55 (s, 1H, Ph-H) 7.96 (br s, 1H, N^6-H), 8.21 and 8.34 (2 × s, 2H, C2-H and C8-H); ESI-MS *m/z* 440.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*o*,*p*-dichlorophenyl)ethyl]adenosine (51b): ^{1}H NMR δ 3.00 (t, 2H, C2″-H), 3.55 and 3.65 (2 × m, 4H, C5′-$H_{a,b}$, and C1″-H), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.08 (br s, 1H, O*H*), 5.34 (br s, 1H, O*H*,), 5.43 (br s, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.78 (br s, 2H, C2-NH_2), 7.33−7.41 (band, 3H, N^6-H and Ph-H), 7.55 (d, 2H, Ph-H), 7.90 (br s, 1H, C8-H); ESI-MS *m/z* 455.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*o*-methoxyphenyl)ethyl]adenosine (52b): ^{1}H NMR δ 2.90 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.79 (s, 3H, Ph-OCH_3), 3.90 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.16 (d, 1H, O*H*), 5.34 (d, 1H, O*H*), 5.45 (m, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.81 (br s, 2H, C2-NH_2), 6.89 (t, 1H, Ph-H), 6.92 (d, 1H, Ph-H), 7.20 (m, 3H, N^6-H and Ph-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 417.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*m*-methoxyphenyl)ethyl]adenosine (53b): ^{1}H NMR δ 2.90 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.79 (s, 3H, Ph-OCH_3), 3.90 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.16 (d, 1H, O*H*), 5.34 (d, 1H, O*H*), 5.45 (m, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.81 (br s, 2H, C2-NH_2), 6.89 (t, 1H, Ph-H), 6.92 (d, 1H, Ph-H), 7.20 (m, 3H, N^6-H and Ph-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 417.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-methoxyphenyl)ethyl]adenosine (54b): ^{1}H NMR δ 2.85 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$, and C1″-H), 3.72 (s, 3H, Ph-OCH_3), 3.93 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.09 (d, 1H, O*H*), 5.34 (d, 1H, O*H*), 5.42 (m, 1H, O*H*), 5.72 (d, 1H, C1′-H), 5.78 (br s, 2H, C2-NH_2), 6.84 (d, 2H, Ph-H), 7.18 (d, 2H, Ph-H), 7.29 (br s, 1H, N^6-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 417.1 $(M + H^+)^+$.

***N*^{6}-[2″-(*p*-Hydroxy-*m*-methoxyphenyl)ethyl]adenosine (56a):** ^{1}H NMR δ 2.78 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.71 (s, 3H, Ph-OCH_3), 3.95 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.21 (d, 1H, O*H*), 5.44 (m, 2H, O*H*), 5.87 (d, 1H, C1′-H), 6.61 (d, 1H, Ph-H), 6.66 (d, 1H, Ph-H), 6.78 (s, 2H, Ph-H), 7.90 (br s, 1H, N^6-H), 8.23 and 8.34 (2 × s, 1H, C2-H and C8-H), 8.72 (s, 1H, Ph-O*H*); ESI-MS *m/z* 418.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-hydroxy-*m*-methoxyphenyl)ethyl]adenosine (56b): ^{1}H NMR δ 2.72 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.71 (s, 3H, Ph-OCH_3), 3.90 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.50 (dd, 1H, C2′-H), 5.13 (d, 1H, O*H*), 5.39 (d, 1H, O*H*), 5.44 (br s, 1H, O*H*), 5.71 (d, 1H, C1′-H), 5.84 (br s, 2H, C2-NH_2), 6.61−6.69 (band, 2H, Ph-H), 6.80 (br s, 1H, Ph-H), 7.31 (br s, 1H, N^6-H), 7.90 (s, 1H, C8-H), 8.71 (s, 1H, Ph-O*H*); ESI-MS *m/z* 433.2 $(M + H^+)^+$.

***N*^{6}-[2″-(*m*-Hydroxy-*p*-methoxyphenyl)ethyl]adenosine (57a):** ^{1}H NMR δ 2.75 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.70 (s, 3H, Ph-OCH_3), 3.95 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.21 (d, 1H, O*H*), 5.44 (m, 1H, O*H*), 5.48 (d, 1H, O*H*), 5.87 (d, 1H, C1′-H), 6.59 (d, 2H, Ph-H), 6.68 (s, 1H, Ph-H), 6.79 (d, 2H, Ph-H), 7.90 (br s, 1H, N^6-H), 8.23 and 8.34 (2 × s, 1H, C2-H and C8-H), 8.88 (s, 1H, Ph-O*H*); ESI-MS *m/z* 418.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*m*-hydroxy-*p*-methoxyphenyl)ethyl]adenosine (57b): ^{1}H NMR δ 2.72 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.71 (s, 3H, Ph-OCH_3), 3.90 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.50 (dd, 1H, C2′-H), 5.13 (d, 1H, O*H*), 5.39 (d, 1H, O*H*), 5.44 (br s, 1H, O*H*), 5.71 (d, 1H, C1′-H), 5.84 (br s, 2H, C2-NH_2), 6.61 (d, 1H, Ph-H), 6.70 (s, 1H, Ph-H), 6.80 (d, 1H, Ph-H), 7.31 (br s, 1H, N^6-H), 7.91 (br s, 1H, C8-H), 8.86 (s, 1H, Ph-O*H*); ESI-MS *m/z* 433.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-aminophenyl)ethyl]adenosine (58b): ^{1}H NMR δ 2.67 (t, 2H, C2″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 4.85 (br s, 2H, Ph-NH_2), 5.09 (d, 1H, OH), 5.36 (d, 1H, OH), 5.48 (m, 1H, OH), 5.71 (d, 1H, C1′-H), 5.81

(br s, 2H, C2-NH_2), 6.48 (d, 2H, Ph-H), 6.90 (d, 2H, Ph-H), 7.35 (s, 1H, N^6-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 402.2 $(M + H^+)^+$.

***N*^{6}-[2″-(*p*-Nitrophenyl)ethyl]adenosine (59a):** ^{1}H NMR δ 3.07 (t, 2H, C2″-H), 3.54 and 3.65 (2 × m, 2H, C5′-$H_{a,b}$), 3.76 (br d, 2H, C1″-H), 3.95 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.59 (dd, 1H, C2′-H), 5.20 (d, 1H, OH), 5.39 (dd, 1H, OH), 5.44 (d, 1H, OH), 5.87 (d, 1H, C1′-H), 7.52 (d, 2H, Ph-H), 8.01 (br s, 1H, N^6-H), 8.14 (d, 2H, Ph-H), 8.23 and 8.34 (2 × s, 1H, C2-H and C8-H); ESI-MS *m/z* 417.1 $(M + H^+)^+$.

2-Amino-*N*^{6}-[2″-(*p*-nitrophenyl)ethyl]adenosine (59b): ^{1}H NMR δ 3.04 (t, 2H, C2″-H), 3.56 and 3.65 (2 × m, 2H, C5′-$H_{a,b}$), 3.76 (br s, 2H, C1″-H), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.11 (d, 1H, OH), 5.36 (d, 1H, OH), 5.44 (s, 1H, OH), 5.72 (d, 1H, C1′-H), 5.90 (br s, 2H, C2-NH_2), 7.50 (br s, 1H, N^6-H), 7.55 (d, 2H, Ph-H), 7.93 (s, 1H, C8-H), 8.14 (d, 2H, Ph-H); ESI-MS *m/z* 432.2 $(M + H^+)^+$.

6-Chloro-2-iodopurine 9-Riboside (62b). As described by Matsuda et al.[29] for the iodination of 9-(2′,3′,5′-triacetyl-1-β-D-ribofuranosyl)-2-amino-6-chloropurine (**61**), fresh isoamyl nitrite (1.45 mL, 10.81 mmol) was added to a mixture of **1b′** (1.0 g, 3.32 mmol), CuI (670 mg, 3.52 mmol), CH_2I_2 (2.67 mL, 33.2 mmol), and I_2 (842 mg, 3.32 mmol) in dry THF (10.0 mL) at ambient temperature and under argon. The reaction mixture was refluxed for 30 min, cooled to ambient temperature, filtered to remove insolubles, and then adsorbed onto silica gel via rotary evaporation. The product was purified via flash chromatography. Iodine was first eluted from the column with 100% $CHCl_3$ and then compound **62b** was eluted with 10% MeOH in $CHCl_3$ in 56% yield: ^{1}H NMR δ 3.58 and 3.70 (2 × m, 2H, C5′-$H_{a,b}$), 3.98 (m, 1H, C4′-H), 4.15 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 5.04 (t, 1H, O*H*), 5.25 (d, 1H, O*H*), 5.56 (d, 1H, O*H*), 5.95 (d, 1H, C1′-H), 8.87 (s, 1H, C8-H); ESI-MS *m/z* 413.0 $(M + H^+)^+$.

Synthesis of 2-Iodoadenosine (1c′) from 62b. As described by Matsuda et al.[29] for the ammoniaolysis of **62a**, ammonia gas was condensed into a solution of **62b** (100 mg, 0.24 mmol) in MeOH (10.0 mL) at 0 °C in a pressure tube. The reaction was heated to 60 °C for 18 h and then chilled to 0 °C in order to safely open the tube. The yellow solution was adsorbed onto silica gel via rotary evaporation and purified via flash chromatography. Compound **1c′** was eluted with 15% MeOH in $CHCl_3$ to yield 87 mg of the desired product (94%): ^{1}H NMR δ 3.54 and 3.64 (2 × m, 2H, C5′-$H_{a,b}$), 3.94 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.54 (dd, 1H, C2′-H), 5.04 (t, 1H, O*H*), 5.20 (t, 1H, O*H*), 5.47 (d, 1H, O*H*), 5.80 (d, 1H, C1′-H), 7.74 (s, 2H, N^6-H), 8.30 (s, 1H, C8-H); ESI-MS *m/z* 394.0 $(M + H^+)^+$.

General Procedure for the Preparation of N^2-Substituted Adenosine Analogues (2a−59a and 2b−59b) from 1c′. In parallel fashion and with minor modifications, the method of Marumoto et al. was employed.[25] To a solution of **1c′** (5.0 mg, 0.013 mmol) in 2-methoxyethanol (100 μL) was added the appropriate amine or amine hydrochloride (0.25 mmol). For reactions requiring the use of a hydrochloride, *i*-Pr_2-EtN (44 μL, 0.25 mmol) was added. The reactions were refluxed for 24 h and then allowed to cool to ambient temperature. Without further workup, the homogeneous reaction mixtures were purified via preparative HPLC method B, except for compounds **10c**−**15c** which were purified by preparative HPLC method C and **16c**−**20c** which were purified by preparative HPLC method A, to give the desired products in 40−85% yield.

2-(*n*-Butylamino)adenosine (6c): ^{1}H NMR (DMSO-d_6 + TFD) δ 0.88 (m, 2H, C4″-H), 1.34 (m, 2H, C3″-H), 1.52 (m, 2H, C2″-H), 3.32 (m, 2H, C1″-H), 3.25 and 3.40 (2 × m, 2H, C5′-$H_{a,b}$), 3.97 (m, 1H, C4′-H), 4.18 (dd, 1H, C3′-H), 4.54 (dd, 1H, C2′-H), 5.81 (d, 1H, C1′-H), 8.33 (s, 1H, C8-H); ESI-MS *m/z* 339.2 $(M + H^+)^+$.

2-(*n*-Amylamino)adenosine (7c): ^{1}H NMR δ 1.41 (t, 3H, C5″-H), 1.49 (p, 2H, C4″-H), 1.63 (p, 2H, C3″-H), 1.86 (p, 2H, C2″-H), 3.50 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.11 (m, 2H, C3′-H and C1"-H), 4.60 (dd, 1H, C2′-H), 5.20 (br s, 3H, O*H*), 5.70 (d, 1H, C1′-H), 6.12 (s, 1H, C2-N*H*), 6.66 (br s, 2H, N^6-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 353.2 $(M + H^+)^+$.

2-[(3″-Hydroxypropyl)amino]adenosine (11c): ^{1}H NMR δ 1.64 (p, 2H, C2″-H), 3.27 (m, 2H, C1″-H), 3.46 (m, 2H, C3″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.87 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.56 (dd, 1H, C2′-H), 5.71 (d, 1H, C1′-H), 6.14 (br s, 1H, C2-N*H*), 6.73 (br s, 2H, N^6-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 341.2 $(M + H^+)^+$.

2-[(2″-Hydroxypropyl)amino]adenosine (12c): ^{1}H NMR δ 1.04 (d, 3H, C3″-H), 3.11 and 3.25 (2 × m, 2H, C1″-$H_{a,b}$), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.76 (m, 1H, C2″-H), 3.87 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.56 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 5.94 (br s, 1H, C2-N*H*), 6.79 (br s, 2H, N^6-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 341.2 $(M + H^+)^+$.

2-[(2″,3″-Dihydroxypropyl)amino]adenosine (13c): ^{1}H NMR δ 3.12−3.62 (band, 7H, C5′-$H_{a,b}$, C1″-$H_{a,b}$, C2″-H, and C3″-$H_{a,b}$), 3.87 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.55 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 5.91 (br s, 1H, C2-N*H*), 6.83 (br s, 2H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS *m/z* 357.1 $(M + H^+)^+$.

2-[(4″-Hydroxybutyl)amino]adenosine (14c): ^{1}H NMR δ 1.44 (m, 2H, C2"-H), 1.49 (m, 2H, C3″-H), 3.21 (m, 2H, C1″-H), 3.38 (m, 2H, C4″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.87 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.58 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.15 (br s, 1H, C2-N*H*), 6.70 (br s, 2H, N^6-H), 7.88 (s, 1H, C8-H); ESI-MS *m/z* 355.2 $(M + H^+)^+$.

2-[(5″-Hydroxypentyl)amino]adenosine (15c): ^{1}H NMR δ 1.32 (m, 2H, C3″-H), 1.42 (m, 2H, C2″-H), 1.49 (m, 2H, C4″-H), 3.21 (m, 2H, C1″-H), 3.38 (m, 2H, C5″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.87 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.58 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.13 (br s, 1H, C2-N*H*), 6.70 (br s, 2H, N^6-H), 7.88 (s, 1H, C8-H); ESI-MS *m/z* 369.2 $(M + H^+)^+$.

2-[(3″-Aminopropyl)amino]adenosine (17c): ^{1}H NMR δ 1.39 (m, 2H, C2″-H), 2.76 (t, 2H, C3″-H), 3.26 (m, 2H, C1″-H), 3.52 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.87 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.55 (dd, 1H, C2′-H), 5.71 (d, 1H, C1′-H), 6.42 (t, 1H, C2-N*H*), 6.81 (br s, 2H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS *m/z* 340.2 $(M + H^+)^+$.

2-[(2″-Hydroxy-3″-aminopropyl)amino]adenosine (18c): ^{1}H NMR δ 2.80 (m, 2H, C3″-H), 3.30 (m, 2H, C1″-H), 3.52 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.80 (br s, 1H, C2″-H), 3.86 (m, 1H, C4′-H), 4.11 (m, 1H, C3′-H), 4.55 (m, 1H, C2′-H), 5.70 (m, 1H, C1′-H), 6.20 (t, 1H, C2-N*H*), 6.81 (br s, 2H, N^6-H), 7.92 (s, 1H, C8-H); ESI-MS *m/z* 356.2 $(M + H^+)^+$.

2-[(5″-Aminopropyl)amino]adenosine (20c): ^{1}H NMR δ 1.33 (m, 2H, C3″-H), 1.57 (m, 4H, C2″-H and C4″-H), 2.70 (m, 2H, C5″-H), 3.20 (m, 2H, C1″-H), 3.52 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.10 (m, 1H, C3′-H), 4.57 (m, 1H, C2′-H), 5.72 (m, 1H, C1′-H), 6.20 (t, 1H, C2-N*H*), 6.75 (br s, 2H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS *m/z* 368.2 $(M + H^+)^+$.

2-[(Cyclohexylmethyl)amino]adenosine (23c): ^{1}H NMR δ 1.32−1.86 (band, 11H, cyclohexyl-H) 3.08 (m, 2H, N^2-CH_2), 3.51 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.59 (dd, 1H, C2′-H), 5.20 (br s, 3H, O*H*), 5.70 (d, 1H, C1′-H), 6.09 (br s, 1H, C2-N*H*), 6.63 (br s, 2H, N^6-H), 7.92 (s, 1H, C8-H); ESI-MS *m/z* 379.2 $(M + H^+)^+$.

2-(Cycloheptylamino)adenosine (24c): ^{1}H NMR δ 1.36−1.86 (band, 12H, cycloheptyl-H) 3.51 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 3.89 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.39 (m, 2H, C1"-H), 4.59 (dd, 1H, C2′-H), 5.20 (br s, 3H, O*H*), 5.70 (d, 1H, C1′-H), 5.88 (d, 1H, C2-N*H*), 6.62 (br s, 2H, N^6-H), 7.86 (s, 1H, C8-H); ESI-MS *m/z* 379.2 $(M + H^+)^+$.

2-(Tetrahydrofurfurylamino)adenosine (25c): ^{1}H NMR δ 1.80−1.99 (band, 4H, C3″- and C4″-H), 3.51 and 3.62 (2 × m, 4H, C5′-$H_{a,b}$ and C5″-H), 3.75 (m, 2H, C1"-H), 3.87 (m, 1H, C4′-H), 3.95 (m, 1H, C2"-H), 4.11 (dd, 1H, C3′-H), 4.43 (m, 2H, N^2-CH_2), 4.56 (dd, 1H, C2′-H), 5.72 (d, 1H, C1′-H), 6.58 (t, 1H, C2-N*H*), 6.80 (br s, 2H, N^6-H), 7.92 (s, 1H, C8-H); ESI-MS *m/z* 367.2 $(M + H^+)^+$.

2-(Furfurylamino)adenosine (26c): ^{1}H NMR δ 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.75 (m, 1H, N^2-CH_2), 3.87 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.43 (t, 2H, C1″-H), 4.56 (dd, 1H, C2′-H), 5.72 (d, 1H, C1′-H), 6.21 (d, 1H, C3″-H), 6.34 (t, 1H,

C4″-H), 6.58 (t, 1H, C2-N*H*), 6.80 (br s, 2H, N^6-H), 7.51 (s, 1H, C5″-H), 7.92 (s, 1H, C8-H); ESI-MS *m/z* 363.2 $(M + H^+)^+$.

2-[(*o*-Methylbenzyl)amino]adenosine (29c): ^{1}H NMR δ 2.30 (s, 3H, Ph-CH_3), 3.45 and 3.57 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.45 (d, 2H, N^2-CH_2), 4.53 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.52 (t, 1H, C2-N*H*), 6.71 (br s, 2H, N^6-H), 7.08 (m, 3H, Bz-H), 7.27 (d, 1H, Bz-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 387.2 $(M + H^+)^+$.

2-[(*m*-Methylbenzyl)amino]adenosine (30c): ^{1}H NMR δ 2.26 (s, 3H, Ph-CH_3), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.45 (m, 2H, N^2-CH_2), 4.53 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.63 (t, 1H, C2-N*H*), 6.71 (br s, 2H, N^6-H), 6.98 (d, 1H, Ph-H), 7.13 (m, 3H, Ph-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 387.2 $(M + H^+)^+$.

2-[(*p*-Methylbenzyl)amino]adenosine (31c): ^{1}H NMR δ 2.28 (s, 3H, Ph-CH_3), 3.50 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.46 (m, 2H, N^2-CH_2), 4.57 (dd, 1H, C2′-H), 5.75 (d, 1H, C1′-H), 6.64 (t, 1H, C2-N*H*), 6.75 (br s, 2H, N^6-H), 7.10 (d, 2H, Ph-H), 7.25 (d, 2H, Ph-H), 7.93 (s, 1H, C8-H); ESI-MS *m/z* 387.2 $(M + H^+)^+$.

2-[(*o*-Chlorobenzyl)amino]adenosine (32c): ^{1}H NMR δ 3.47 and 3.58 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.53 (dd, 1H, C2′-H), 4.57 (m, 2H, N^2-CH_2), 5.70 (d, 1H, C1′-H), 6.70 (t, 1H, C2-N*H*), 6.77 (br s, 2H, N^6-H), 7.36 (m, 2H, Ph-H), 7.83 (m, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 407.1 $(M + H^+)^+$.

2-[(*m*-Chlorobenzyl)amino]adenosine (33c): ^{1}H NMR δ 3.46 and 3.58 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.47 (d, 2H, N^2-CH_2), 4.53 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.76 (br s, 2H, N^6-H), 6.82 (t, 1H, C2-N*H*), 7.22-7.30 (band, 4H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 407.1 $(M + H^+)^+$.

2-[(*p*-Chlorobenzyl)amino]adenosine (34c): ^{1}H NMR δ 3.46 and 3.58 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.44 (d, 2H, N^2-CH_2), 4.53 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.73 (br s, 2H, N^6-H), 6.78 (t, 1H, C2-N*H*), 7.31 (d, 2H, Ph-H), 7.34 (d, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 407.1 $(M + H^+)^+$.

2-[(*o*-Methoxybenzyl)amino]adenosine (35c): ^{1}H NMR δ 3.46 and 3.58 (2 × m, 2H, C5′-$H_{a,b}$), 3.81 (s, 3H, Ph-OCH_3), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.45 (m, 2H, N^2-CH_2), 4.52 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.33 (t, 1H, C2-N*H*), 6.73 (br s, 2H, N^6-H), 6.84 (t, 1H, Ph-H), 6.94 (d, 1H, Ph-H), 7.18 (m, 2H, Ph-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 403.2 $(M + H^+)^+$.

2-[(*p*-Methoxybenzyl)amino]adenosine (36c): ^{1}H NMR δ 3.50 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.70 (s, 3H, Ph-OCH_3), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.39 (d, 2H, N^2-CH_2), 4.54 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 6.59 (t, 1H, C2-N*H*), 6.71 (br s, 2H, N^6-H), 6.82 (d, 2H, Ph-H), 7.23 (d, 2H, Ph-H), 7.89 (s, 1H, C8-H); ESI-MS *m/z* 403.2 $(M + H^+)^+$.

2-[[2″-(*p*-Methylphenyl)ethyl]amino]adenosine (44c): ^{1}H NMR δ 2.25 (s, 3H, Ph-CH_3), 2.77 (t, 2H, C2″-H), 3.40 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.12 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 5.30 (br s, 3H, O*H*), 5.73 (d, 1H, C1′-H), 6.13 (t, 1H, C2-N*H*), 6.73 (br s, 2H, N^6-H), 7.08 (d, 2H, Ph-H), 7.13 (d, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 401.2 $(M + H^+)^+$.

2-[[2″-(*p*-Hydroxyphenyl)ethyl]amino]adenosine (46c): ^{1}H NMR δ 2.71 (t, 2H, C2″-H), 3.41 (m, 2H, C1″-H), 3.51 and 3.60 (2 × m, 2H, C5′-$H_{a,b}$), 3.90 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.62 (dd, 1H, C2′-H), (br s, 3H, O*H*), 5.72 (d, 1H, C1′-H), 6.15 (br s, 1H, C2-N*H*), 6.68 (d, 2H, Ph-H), 6.75 (br s, 2H, N^6-H), 7.05 (d, 2H, Ph-H), 7.92 (s, 1H, C8-H), 9.30 (s, 1H, Ph-O*H*); ESI-MS *m/z* 403.2 $(M + H^+)^+$.

2-[[2″-(*o*-Chlorophenyl)ethyl]amino]adenosine (48c): ^{1}H NMR δ 2.97 (t, 2H, C2″-H), 3.45 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.12 (dd, 1H, C3′-H), 4.58 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.75 (d, 1H, C1′-H), 6.29 (m, 1H, C2-N*H*), 6.73 (br s, 2H, N^6-H), 7.25 (m, 2H, Ph-H), 7.39 (m, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 421.1 $(M + H^+)^+$.

2-[[2″-(*m*-Chlorophenyl)ethyl]amino]adenosine (49c): ^{1}H NMR δ 2.85 (t, 2H, C2″-H), 3.44 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.73 (d, 1H, C1′-H), 6.25 (m, 1H, C2-N*H*), 6.75 (br s, 2H, N^6-H), 7.25 (m, 2H, Ph-H), 7.30 (m, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 421.1 $(M + H^+)^+$.

2-[[2″-(*p*-Chlorophenyl)ethyl]amino]adenosine (50c): ^{1}H NMR δ 2.82 (t, 2H, C2″-H), 3.43 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.73 (d, 1H, C1′-H), 6.24 (m, 1H, C2-N*H*), 6.75 (br s, 2H, N^6-H), 7.27 (d, 2H, Ph-H), 7.32 (d, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 421.1 $(M + H^+)^+$.

2-[[2″-(*o,p*-Dichlorophenyl)ethyl]amino]adenosine (51c): ^{1}H NMR δ 2.95 (t, 2H, C2″-H), 3.44 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.12 (dd, 1H, C3′-H), 4.57 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.74 (d, 1H, C1′-H), 6.32 (m, 1H, C2-N*H*), 6.74 (br s, 2H, N^6-H), 7.34 (d, 2H, Ph-H), 7.40 (d, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 455.1 $(M + H^+)^+$.

2-[[2″-(*o*-Methoxyphenyl)ethyl]amino]adenosine (52c): ^{1}H NMR δ 2.81 (t, 2H, C2″-H), 3.42 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.79 (s, 3H, Ph-OCH_3), 3.88 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.58 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.73 (d, 1H, C1′-H), 6.08 (m, 1H, C2-N*H*), 6.72 (m, 2H, N^6-H), 6.85 (t, 1H, Ph-H), 6.94 (d, 1H, Ph-H), 7.16 (m, 2H, Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m/z* 417.2 $(M + H^+)^+$.

2-[[2″-(*m*-Methoxyphenyl)ethyl]amino]adenosine (53c): ^{1}H NMR δ 2.80 (t, 2H, C2″-H), 3.44 (m, 2H, C1″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.73 (s, 3H, Ph-OCH_3), 3.88 (m, 1H, C4′-H), 4.12 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.73 (d, 1H, C1′-H), 6.16 (m, 1H, C2-N*H*), 6.74 (m, 4H, N^6-H and Ph-H), 6.81 (m, 2H, Ph-H), 7.19 (t, 1H, Ph-H), 7.93 (s, 1H, C8-H); ESI-MS *m/z* 417.2 $(M + H^+)^+$.

General Procedure for the Preparation of N^6,N^2-Disubstituted Adenosine Analogues (69−82) from 62a. To a solution of **62a** (5.0 mg, 0.009 mmol) in 2-methoxyethanol (250 μL) were added the appropriate amine or amine hydrochloride (0.01 mmol) targeted for the 6-position and then $CaCO_3$ (60.0 mg, 0.06 mmol). The reaction was heated at 60 °C for 18 h and then allowed to cool to ambient temperature. The amine or amine hydrochloride (0.184 mmol) targeted for the 2-position was then added and the reaction was refluxed for 24 h. For reactions with amine hydrochlorides targeted for the 2-position, *i*-Pr_2EtN (32 μL, 0.184 mmol) was added prior to heating. After the reactions were cooled to ambient temperature, water (100 μL) was added to the reaction to dissolve any insoluble salts and, without further workup, the reactions were purified via preparative HPLC method B to give the desired products in 40−70% yield.

General Procedure for the Preparation of N^6,N^2-Disubstituted Adenosine Analogues (69−82) from 62b. To a solution of **62b** (5.0 mg, 0.012 mmol) in 2-methoxyethanol (100 μL) were added the appropriate amine or amine hydrochloride (0.013 mmol) targeted for the 6-position and then *i*-Pr_2EtN (6.3 μL, 0.036 mmol for free amines; 8.4 μL, 0.048 mmol for hydrochlorides). The reaction was heated at 60 °C for 18 h and then allowed to cool to ambient temperature. The amine or amine hydrochloride (0.24 mmol) targeted for the 2-position was then added and the reaction was refluxed for 24 h. For reactions with amine hydrochlorides targeted for the 2-position, *i*-Pr_2EtN (42 μL, 0.24 mmol) was added prior to heating. The reactions were cooled to ambient temperature and, without further workup, the reactions were purified via preparative HPLC method B to give the desired products in 50−80% yield. Intermediates **63b**−**68b** were isolated via flash chromatography, in 90−95% yield, for the purpose of characterization.

2-Iodo-N^6-(2″-phenylethyl)adenosine (63b): ^{1}H NMR δ 2.89 (t, 2H, C2″-H), 3.51 and 3.62 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.93 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.53 (dd, 1H, C2′-H), 5.03 (t, 1H, O*H*), 5.21 (d, 1H, O*H*), 5.47 (d, 1H, O*H*), 5.78 (d, 1H, C1′-H), 7.17−7.30 (band, 5H, Ph-H), 8.29 (m, 2H, N^6-H and C8-H); ESI-MS *m/z* 498.1 $(M + H^+)^+$.

2-Iodo-N^6-[2″-(*p*-hydroxyphenyl)ethyl]adenosine (64b): ^{1}H NMR δ 2.76 (t, 2H, C2″-H), 3.53 and 3.62 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.93 (m, 1H, C4′-H), 4.11 (dd, 1H,

C3′-H), 4.52 (dd, 1H, C2′-H), 5.03 (t, 1H, O*H*), 5.21 (d, 1H, O*H*), 5.48 (d, 1H, O*H*), 5.79 (d, 1H, C1′-H), 6.66 (d, 2H, Ph-H), 7.04 (dd, 2H, Ph-H), 8.25 (t, 1H, N^6-H), 8.28 (s, 1H, C8-H), 9.18 (s, 1H, Ph-O*H*); ESI-MS *m*/*z* 514.1 $(M + H^+)^+$.

2-Iodo-N^6-[2″-(*p*-aminophenyl)ethyl]adenosine (65b): ^{1}H NMR δ 2.69 (t, 2H, C2″-H), 3.53 and 3.62 (2 × m, 4H, C5′-$H_{a,b}$ and C1″-H), 3.93 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 4.86 (s, 2H, Ph-NH_2), 5.03 (t, 1H, O*H*), 5.21 (d, 1H, O*H*), 5.45 (d, 1H, O*H*), 5.79 (d, 1H, C1′-H), 6.48 (d, 2H, Ph-H), 6.89 (dd, 2H, Ph-H), 8.21 (t, 1H, N^6-H), 8.27 (s, 1H, C8-H); ESI-MS *m*/*z* 513.1 $(M + H^+)^+$.

2-Iodo-N^6-*n*-butyladenosine (66b): ^{1}H NMR δ 0.88 (t, 3H, N^6-C4″-H), 1.30 (m, 2H, N^6-C3″-H), 1.52 (m, 2H, N^6-C2″-H), 3.35 (m, 2H, N^6-C1"-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.91 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 5.04 (t, 1H, O*H*), 5.22 (d, 1H, O*H*), 5.47 (d, 1H, O*H*), 5.79 (d, 1H, C1′-H), 8.21 (m, 1H, N^6-N*H*), 8.28 (s, 1H, C8-H); ESI-MS *m*/*z* 450.1 $(M + H^+)^+$.

2-Iodo-N^6-tetrahydrofurfuryladenosine (67b): ^{1}H NMR δ 1.60−1.89 (band, 4H, C3″-H and C4″-H), 3.51−4.02 (band, 7H, N^6-CH_2, C4′-H, C5′-$H_{a,b}$, and C5″-H), 4.10 (dd, 1H, C3′-H), 4.51 (dd, 1H, C2′-H), 5.05 (t, 1H, O*H*), 5.22 (d, 1H, O*H*), 5.48 (d, 1H, O*H*), 5.80 (d, 1H, C1′-H), 8.15 (m, 1H, N^6-H), 8.31 (s, 1H, C8-H); ESI-MS *m*/*z* 478.1 $(M + H^+)^+$.

2-Iodo-N^6-(1″-naphthylmethyl)adenosine (68b): ^{1}H NMR δ 3.53 and 3.63 (2 × m, 2H, C5′-$H_{a,b}$), 3.92 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.52 (dd, 1H, C2′-H), 5.04 (t, 1H, O*H*), 5.08 (br s, 2H, N^6-CH_2), 5.22 (d, 1H, O*H*), 5.48 (d, 1H, O*H*), 5.80 (d, 1H, C1′-H), 7.45 (m, 2H, naphthyl-H), 7.55 (m, 2H, naphthyl-H), 7.83 (d, 1H, naphthyl-H), 7.93 (d, 1H, naphthyl-H), 8.29 (d, 1H, naphthyl-H), 8.33 (s, 1H, C8-H), 8.87 (m, 1H, N^6-H); ESI-MS *m*/*z* 534.1 $(M + H^+)^+$.

***N*^{6}-(2″-Phenylethyl)-2-[(2″-phenylethyl)amino]adenosine (69):** ^{1}H NMR δ 2.82 (t, 2H, N^2-C2″-H), 2.92 (t, 2H, N^6-C2″-H), 3.47−3.68 (band, 6H, N^2-Cl″-H, N^6-C1″-H and C5′-$H_{a,b}$), 3.90 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 5.13 (br s, 2H, O*H*), 5.41 (br s, 1H, O*H*), 5.73 (d, 1H, C1′-H), 6.38 (br s, 1H, C2-N*H*), 7.15−7.29 (band, 10H, Ph-H), 7.47 (br s, 1H, N^6-H), 7.92 (s, 1H, C8-H); ESI-MS *m*/*z* 491.2 $(M + H^+)^+$.

***N*^{6}-[2″-(*p*-Hydroxyphenyl)ethyl]-2-[[2″-(*p*-hydroxyphenyl)ethyl]amino]adenosine (70):** ^{1}H NMR δ 2.66 (t, 2H, N^2-C2″-H), 2.76 (t, 2H, N^6-C2″-H), 3.38−3.63 (band, 6H, N^2-C1"-H, N^6-C1″-H and C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.73 (d, 1H, C1′-H), 6.38 (br s, 1H, C2-N*H*), 6.62 (d, 4H, Ph-H), 7.01 (d, 4H, Ph-H), 7.45 (br s, 1H, N^6-H), 7.95 (s, 1H, C8-H), 9.30 (br s, 2H, Ph-O*H*); ESI-MS *m*/*z* 523.2 $(M + H^+)^+$.

***N*^{6}-[2″-(*p*-Aminophenyl)ethyl]-2-[[2″-(*p*-aminophenyl)ethyl]amino]adenosine (71):** ^{1}H NMR δ 2.67 (t, 2H, N^2-C2″-H), 2.72 (t, 2H, N^6-C2″-H), 3.45−3.68 (band, 6H, N^2-C1″-H, N^6-C1″-H and C5′-$H_{a,b}$), 3.90 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.61 (dd, 1H, C2′-H), 4.90 (br s, 4H, Ph-NH_2), 5.13 (br s, 2H, O*H*), 5.40 (d, 1H, O*H*), 5.72 (d, 1H, C1′-H), 6.25 (br s, 1H, C2-N*H*), 6.51 (d, 4H, Ph-H), 6.92 (d, 4H, Ph-H), 7.45 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS *m*/*z* 521.3 $(M + H^+)^+$.

***N*^{6}-[2″-(*p*-Hydroxyphenyl)ethyl]-2-[(2″-phenylethyl)amino]adenosine (72):** ^{1}H NMR δ 2.72 (t, 2H, N^2-C2″-H), 2.88 (t, 2H, N^6-C2″-H), 3.35−3.66 (band, 6H, N^2-C1″-H, N^6-C1″-H and C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.14 (dd, 1H, C3′-H), 4.60 (dd, 1H, C2′-H), 5.25 (br d, 3H, O*H*), 5.75 (d, 1H, C1′-H), 6.36 (br s, 1H, C2-N*H*), 6.64 (d, 2H, Ph-H), 7.01 (d, 2H, Ph-H), 7.18−7.26 (band, 5H, Ph-H), 7.45 (br s, 1H, N^6-H), 7.93 (s, 1H, C8-H), 9.28 (br s, 1H, Ph-O*H*); ESI-MS *m*/*z* 507.2 $(M + H^+)^+$.

***N*^{6}-(2″-Phenylethyl)-2-[[2″-(*p*-hydroxyphenyl)ethyl]amino]adenosine (73):** ^{1}H NMR δ 2.69 (t, 2H, N^2-C2″-H), 2.88 (t, 2H, N^6-C2″-H), 3.32−3.63 (band, 6H, N^2-C1″-H, N^6-C1″-H, and C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.58 (dd, 1H, C2′-H), 5.25 (br s, 3H, O*H*), 5.74 (d, 1H, C1′-H), 6.35 (br s, 1H, C2-N*H*), 6.63 (d, 2H, Ph-H), 7.01 (d, 2H, Ph-H), 7.18−7.26 (band, 5H, Ph-H), 7.48 (br s, 1H, N^6-H), 7.93 (s, 1H, C8-H), 9.20 (br s, 1H, Ph-O*H*); ESI-MS *m*/*z* 507.2 $(M + H^+)^+$.

***N*^{6}-*n*-Butyl-2-[(3″-hydroxypropyl)amino]adenosine (74):** ^{1}H NMR δ 0.88 (t, 3H, N^6-C4″-H), 1.30 (m, 2H, N^6-C3″-H), 1.53 (m, 2H, N^6-C2″-H), 1.65 (m, 2H, N^2-C2″-H), 3.25−3.63 (band, 6H, N^2-C1″-H, N^6-C1″-H, and C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.44 (t, 1H, C3″-O*H*), 4.55 (dd, 1H, C2′-H), 5.11 (br s, 2H, O*H*), 5.35 (br s, 1H, O*H*), 5.69 (d, 1H, C1′-H), 6.16 (br s, 1H, C2-N*H*), 7.27 (br s, 1H, N^6-H), 7.86 (s, 1H, C8-H); ESI-MS *m*/*z* 397.2 $(M + H^+)^+$.

***N*^{6}-*n*-Butyl-2-[(cyclohexylmethyl)amino]adenosine (75):** ^{1}H NMR δ 0.88−1.71 (band, 18H, N^6-C4″-H, N^6-C3″-H, N^6-C2″-H, and N^2-cyclohexyl-H), 3.08 (m, 2H, N^2-C1″-H), 3.37 (m, 2H, N^6-C1″-H), 3.51 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.10 (dd, 1H, C3′-H), 4.56 (dd, 1H, C2′-H), 5.10 (br s, 2H, O*H*), 5.35 (br s, 1H, O*H*), 5.69 (d, 1H, C1′-H), 6.22 (br s, 1H, C2-N*H*), 7.32 (br s, 1H, N^6-H), 7.85 (s, 1H, C8-H); ESI-MS *m*/*z* 435.3 $(M + H^+)^+$.

***N*^{6}-*n*-Butyl-2-(benzylamino)adenosine (76):** ^{1}H NMR δ 0.84 (t, 3H, N^6-C4″-H), 1.25 (m, 2H, N^6-C3″-H), 1.46 (m, 2H, N^6-C2″-H), 3.33 (m, 2H, N^6-C1″-H), 3.49 and 3.61 (2 × m, 2H, C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.09 (dd, 1H, C3′-H), 4.46 (d, 2H, N^2-CH_2), 4.53 (dd, 1H, C2′-H), 5.12 (br s, 2H, O*H*), 5.34 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 6.85 (br s, 1H, C2-N*H*), 7.16−7.47 (band, 6H, N^6-H and Ph-H), 7.89 (s, 1H, C8-H); ESI-MS *m*/*z* 429.2 $(M + H^+)^+$.

***N*^{6}-Tetrahydrofurfuryl-2-[(3″-hydroxypropyl)amino]adenosine (77):** ^{1}H NMR δ 1.69−1.87 (band, 6H, N^6-C3″-H, N^6-C4″-H, and N^2-C2″-H), 3.29−3.78 (band, 10H, N^6-CH_2, N^6-C5″-H, N^2-C1″-H, N^2-C3″-H and C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.05 (m, 1H, N^6-C2″-H), 4.11 (dd, 1H, C3′-H), 4.45 (br s, 1H, N^2-C3″-O*H*), 4.56 (dd, 1H, C2′-H), 5.14 (br s, 2H, O*H*), 5.38 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 6.25 (br s, 1H, C2-N*H*), 7.19 (br s, 1H, N^6-H), 7.89 (s, 1H, C8-H); ESI-MS *m*/*z* 425.2 $(M + H^+)^+$.

***N*^{6}-Tetrahydrofurfuryl-2-[(cyclohexylmethyl)amino]adenosine (78):** ^{1}H NMR δ 0.88−1.87 (band, 15H, N^6-C3″-H, N^6-C4″-H, and N^2-cyclohexyl-H), 3.07 (m, 2H, N^2-CH_2), 3.39−3.78 (band, 6H, N^6-CH_2, N^6-C5″-H, N^2-C1″-H, N^2-C3″-H and C5′-$H_{a,b}$), 3.86 (m, 1H, C4′-H), 4.05 (m, 1H, N^6-C2″-H), 4.10 (dd, 1H, C3′-H), 4.56 (dd, 1H, C2′-H), 5.11 (br s, 2H, O*H*), 5.37 (br s, 1H, O*H*), 5.69 (d, 1H, C1′-H), 6.25 (br s, 1H, C2-N*H*), 7.17 (br s, 1H, N^6-H), 7.87 (s, 1H, C8-H); ESI-MS *m*/*z* 463.3 $(M + H^+)^+$.

***N*^{6}-Tetrahydrofurfuryl-2-(benzylamino)adenosine (79):** ^{1}H NMR δ 1.52−1.80 (band, 4H, N^6-C3″-H and N^6-C4″-H), 3.51−4.10 (band, 7H, N^6-CH_2, N^6-C5″-H, N^2-CH^2, C3′-H, C4′-H, and C5′-$H_{a,b}$), 4.56 (dd, 1H, C2′-H), 5.11 (br s, 2H, O*H*), 5.37 (br s, 1H, O*H*), 5.70 (d, 1H, C1′-H), 6.98 (br s, 1H, C2-N*H*), 7.11−7.38 (band, 6H, N^6-H and Ph-H), 7.90 (s, 1H, C8-H); ESI-MS *m*/*z* 457.2 $(M + H^+)^+$.

***N*^{6}-(1″-Naphthylmethyl)-2-[(3″-hydroxypropyl)amino]adenosine (80):** ^{1}H NMR δ 1.58 (m, 2H, N^2-C2″-H), 3.22 and 3.27 (2 × m, 4H, N^2-C1″-H and N^2-C3″-H), 3.52 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.38 (t, 1H, C3″-O*H*), 4.58 (dd, 1H, C2′-H), 5.11 (m, 4H, N^6-CH_2 and O*H*), 5.36 (br s, 1H, O*H*), 5.71 (d, 1H, C1′-H), 6.28 (br s, 1H, C2-N*H*), 7.41−7.96 (band, 8H, C8-H and naphthyl-H), 8.22 (br s, 1H, N^6-H); ESI-MS *m*/*z* 481.2 $(M + H^+)^+$.

***N*^{6}-(1″-Naphthylmethyl)-2-[(cyclohexylmethyl)amino]adenosine (81):** ^{1}H NMR δ 1.30−1.80 (band, 11H, N^2-cyclohexyl-H), 2.97 (m, 2H, N^2-CH_2), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.88 (m, 1H, C4′-H), 4.11 (dd, 1H, C3′-H), 4.58 (dd, 1H, C2′-H), 5.12 (m, 4H, N^6-CH_2 and O*H*), 5.38 (br s, 1H, O*H*), 5.71 (d, 1H, C1′-H), 6.26 (br s, 1H, C2-N*H*), 7.41−7.96 (band, 8H, C8-H and naphthyl-H), 8.25 (br s, 1H, N^6-H); ESI-MS *m*/*z* 519.3 $(M + H^+)^+$.

General Procedure for the Preparation of Diaminoalkyl-Functionalized Trityl Resins. To a slurry of trityl chloride resin (0.48 mmol) in CH_2Cl_2 (2.0 mL) were added triethylamine (2.36 mmol) and a diamine (2.36 mmol). The reactions were shaken on a platform shaker for 18 h at ambient temperature. The resins were filtered and washed with CH_2Cl_2 (3 × 3 mL), THF (3 × 3 mL), and CH_2Cl_2 (3 × 3 mL), and then dried in vacuo for 24 h.

General Procedure for the Preparation of Diaminoalkyl Nucleoside-Functionalized Trityl Resins. To a

slurry of diaminoalkyltrityl resin (0.05 mmol) in CH_2Cl_2/EtOH (1:1, 1.0 mL) were added Et_3N (0.24 mmol) and either **1a′** or **1b′** (0.24 mmol). The reactions were heated without stirring at 60 °C for 18 h. After the reactions were cooled to ambient temperature, the resins were filtered and washed with CH_2Cl_2/EtOH (1:1, 3 × 3 mL), EtOH (2 × 3 mL), THF (2 × 3 mL), and CH_2Cl_2 (3 × 3 mL), and then dried in vacuo for 24 h.

General Procedure for the Cleavage of Diaminoalkyl Nucleoside from Trityl Resin. A slurry of diaminoalkyl nucleoside trityl resin in 30% hexafluoro-2-propanol in CH_2Cl_2[30] (2.0 mL) was shaken on a platform shaker for 15 min at ambient temperature. The resins were filtered and then washed with CH_2Cl_2/EtOH (1:1, 3 × 3 mL) and EtOH (3 × 3 mL). The washings were combined with the initial filtrate and stripped to dryness via rotary evaporation to give the desired diaminoalkyl nucleosides, **16a**−**20a** and **16b**−**20b**, in greater than 90% yield and in greater than 95% purity as determined by HPLC and NMR.

2-Amino-*N*^{6}-(2″-aminoethyl)adenosine (16b): ^{1}H NMR δ 2.72 (t, 2H, C2″-H), 3.38 (br s, 2H, C1″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.71 (d, 1H, C1′-H), 5.75 (br s, 2H, C2-NH_2), 7.20 (br s, 1H, N^6-H), 7.90 (s, 1H, C8-H); ESI-MS *m*/*z* 326.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(3″-aminopropyl)adenosine (17b): ^{1}H NMR δ 1.62 (m, 2H, C2″-H), 2.58 (t, 2H, C3″-H), 3.42 (br s, 2H, C1″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.70 (d, 1H, C1′-H), 5.74 (br s, 2H, C2-NH_2), 7.30 (br s, 1H, N^6-H), 7.88 (s, 1H, C8-H); ESI-MS *m*/*z* 340.2 $(M + H^+)^+$.

***N*^{6}-(3″-Amino-2″-hydroxypropyl)adenosine (18a):** ^{1}H NMR δ 2.58 (dd, 2H, C3″-H), 3.41−3.73 (band, 5H, C5′-$H_{a,b}$, C2″-H and C1″-H), 3.95 (m, 1H, C4′-H), 4.13 (dd, 1H, C3′-H), 4.59 (dd, 1H, C2′-H), 5.87 (d, 1H, C1′-H), 7.69 (br s, 1H, N^6-H), 8.20 and 8.34 (2 × s, 1H, C2-H and C8-H); ESI-MS *m*/*z* 341.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(3″-amino-2″-hydroxypropyl)adenosine (18b): ^{1}H NMR δ 2.54 (dd, 2H, C3″-H), 3.41−3.73 (band, 5H, C5′-$H_{a,b}$, C2″-H and C1″-H), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.48 (dd, 1H, C4′-H), 5.70 (d, 1H, C1′-H), 5.81 (br s, 2H, C2-NH_2), 7.19 (br s, 1H, N^6-H), 7.91 (s, 1H, C8-H); ESI-MS *m*/*z* 356.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(4″-aminobutyl)adenosine (19b): ^{1}H NMR δ 1.36 (m, 2H, C3″-H), 1.62 (m, 2H, C2″-H), 2.54 (t, 2H, C4″-H), 3.40 (br s, 2H, C1″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.70 (m, 3H, C1′-H and C2-NH_2), 7.28 (br s, 1H, N^6-H), 7.88 (s, 1H, C8-H); ESI-MS *m*/*z* 354.2 $(M + H^+)^+$.

2-Amino-*N*^{6}-(5″-aminopentyl)adenosine (20b): ^{1}H NMR δ 1.27−1.38 (band, 4H, C3″-H and C4″-H), 1.54 (m, 2H, C2″-H), 2.50 (t, 2H, C4″-H), 3.40 (br s, 2H, C1″-H), 3.51 and 3.62 (2 × m, 2H, C5′-$H_{a,b}$), 3.89 (m, 1H, C4′-H), 4.08 (dd, 1H, C3′-H), 4.49 (dd, 1H, C2′-H), 5.70 (m, 3H, C1′-H and C2-NH_2), 7.25 (br s, 1H, N^6-H), 7.88 (s, 1H, C8-H); ESI-MS *m*/*z* 368.2 $(M + H^+)^+$.

Synthesis of Alkyl-Bridged Bisadenosines 16a′−20a′ and 16b′−20b′. These compounds were either synthesized as described by Agathocleous et al.[32] or isolated as impurities generated from the synthesis of compounds **16a**−**20a** and **16b**−**20b** via Scheme 1.

***N*^{6},*N*$^{6'}$-Ethanediylbis-2-aminoadenosine (16b′):** ^{1}H NMR δ 3.45 and 3.60 (2 × m, 6H, C5′-$H_{a,b}$ and C1″-H), 3.88 (m, 2H, C4′-H), 4.08 (m, 2H, C3′-H), 4.48 (m, 2H, C2′-H), 5.10 (br s, 2H, O*H*), 5.39 (br s, 4H, O*H*), 5.71 (d, 2H, C1′-H), 6.05 (br s, 4H, C2-NH_2), 7.93 (s, 2H, C8-H), 8.06 (br s, 2H, N^6-H); ESI-MS *m*/*z* 591.2 $(M + H^+)^+$.

***N*^{6},*N*$^{6'}$-Propanediylbis-2-aminoadenosine (17b′):** ^{1}H NMR δ 1.88 (br s, 2H, C2″-H), 3.45−3.60 (band, 8H, C5′-$H_{a,b}$ and C1″-H), 3.86 (m, 2H, C4′-H), 4.10 (m, 2H, C3′-H), 4.50 (m, 2H, C2′-H), 5.12 (br s, 2H, O*H*), 5.39 (br s, 4H, O*H*), 5.72 (d, 2H, C1′-H), 6.01 (br s, 4H, C2-NH_2), 7.79 (br s, 2H, N^6-H), 7.93 (s, 2H, C8-H); ESI-MS *m*/*z* 605.3 $(M + H^+)^+$.

***N*^{6},*N*$^{6'}$-2″-Hydroxypropanediylbisadenosine (18a′):** ^{1}H NMR δ 3.50−3.68 (band, 9H, C5′-$H_{a,b}$, C1″-H and C2″-H), 3.96 (m, 2H, C4′-H), 4.13 (m, 2H, C3′-H), 4.61 (m, 2H, C2′-H), 5.19 (d, 2H, O*H*), 5.27 (br s, 1H, C2″-O*H*), 5.39 (m, 2H, O*H*), 5.41 (d, 2H, O*H*), 5.88 (d, 2H, C1′-H), 7.83 (br s, 2H, N^6-H), 8.22 and 8.38 (2 × s, 4H, C2-H and C8-H); ESI-MS *m*/*z* 591.2 $(M + H^+)^+$.

***N*^{6},*N*$^{6'}$-2″-Hydroxypropanediylbis-2-aminoadenosine (18b′):** ^{1}H NMR δ 3.54 and 3.65 (2 × m, 8H, C5′-$H_{a,b}$ and C1″-H), 3.73 (m, 1H, C2″-H), 3.89 (m, 2H, C4′-H), 4.08 (m, 2H, C3′-H), 4.49 (m, 2H, C2′-H), 5.03 (br s, 1H, C2"-O*H*), 5.11 (d, 2H, O*H*), 5.37 (m, 4H, O*H*), 5.73 (d, 2H, C1′-H), 6.25 (br s, 4H, C2-NH_2), 7.80 (br s, 2H, N^6-H), 7.98 (s, 2H, C8-H); ESI-MS *m*/*z* 621.2 $(M + H^+)^+$.

***N*^{6},*N*$^{6'}$-Butanediylbis-2-aminoadenosine (19b′):** ^{1}H NMR δ 1.62 (br s, 4H, C2″-H), 3.39−4.65 (band, 8H, C5′-$H_{a,b}$ and C2″-H), 3.88 (m, 2H, C4′-H), 4.08 (m, 2H, C3′-H), 4.48 (m, 2H, C2′-H), 5.10 (br s, 2H, O*H*), 5.34 (br s, 2H, O*H*), 5.68 (br s, 2H, O*H*), 5.71 (d, 2H, C1′-H), 5.82 (br s, 4H, C2-NH_2), 7.37 (br s, 2H, N^6-H), 7.88 (s, 2H, C8-H); ESI-MS *m*/*z* 619.3 $(M + H^+)^+$.

***N*^{6},*N*$^{6'}$-Pentanediylbis-2-aminoadenosine (20b′):** ^{1}H NMR δ 1.34 (m, 2H, C3″-H), 1.58 (m, 4H, C2″-H), 3.42 (m, 4H, C1″-H), 3.55 and 3.66 (2 × m, 4H, C5′-$H_{a,b}$), 3.88 (m, 2H, C4′-H), 4.08 (m, 2H, C3′-H), 4.48 (m, 2H, C2′-H), 5.10 (br s, 2H, O*H*), 5.36 (br s, 2H, O*H*), 5.65 (br s, 2H, O*H*), 5.71 (d, 2H, C1′-H), 5.78 (br s, 4H, C2-NH_2), 7.34 (br s, 2H, N^6-H), 7.88 (s, 2H, C8-H); ESI-MS *m*/*z* 633.3 $(M + H^+)^+$.

Inhibition Studies. All 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 monitoring absorption at 340 nm using a quartz cuvette. Activity for PGK was measured in the direction of NAD^+ formation, while activity for GAPDH and GPDH was measured in the direction of NADH formation. For the GAPDH assay, fresh glyceraldehyde-3-phosphate was prepared from its diethyl acetal as described by the manufacturer. All compounds tested were dissolved in DMSO-d_6 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 percent of control using the initial velocities measured from 0−2 min. Inhibitor concentration in the cuvette was 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.

D-(−)-3-Phosphoglyceric acid [tri(cyclohexylammonium) salt], adenosine-5′-triphosphate (disodium salt), D-glyceraldehyde-3-phosphate diethyl acetal [di(cyclohexylammonium) salt], dihydroxyacetone phosphate (lithium salt), NAD^+, β-NADH, and rabbit muscle PGK (sulfate-free lyophilized powder) were purchased from Sigma Chemical Co. Recombinant *T. brucei* PGK, GAPDH, and GPDH were expressed and purified as described by Misset et al.[37]

PGK Inhibition Assay. Final concentration of the components in the assay buffer was as follows: 5.0 mM $MgSO_4$, 1.0 mM EDTA, 7.1 mM $NaHCO_3$, 2.0 mM DTT, 5.1 mM 3-PGA, 1.1 mM ATP, 0.42 mM β-NADH, *T. brucei* PGK, and GAPDH (200-fold molar excess of PGK).

GAPDH Inhibition Assay. 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^+, *T. brucei* GAPDH, and 1.87 mM glyceraldehyde-3-phosphate.

GPDH Inhibition Assay. Final concentration of the components in the assay buffer was as follows: 0.60 mM NAD^+, 19.27 mM glycerol-3-phosphate, and *T. brucei* GPDH.

Crystallography. Our crystallization experiments utilized a truncated form of PGK which is made by exchanging the last 34 residues of *T. brucei* glycosomal PGK with the C-terminal 14 amino acids of *T. brucei* cytosolic PGK.[38] It is believed that the highly flexible native C-terminal region of PGK is not conducive to obtaining crystal structures. For the crystallization of this truncated PGK with **46b**, a solution of pure protein was concentrated to 6.0−7.0 mg/mL and mixed with a 100-fold molar excess of **46b**. Crystals of the PGK/**46b** complex were obtained after a few weeks from a reservoir solution containing 0.1 M Tris/HCl (pH = 7.5) and 2.4 M $(NH_4)_2SO_4$ by the hanging drop vapor diffusion method.

One of these crystals ($0.3 \times 0.3 \times 0.02$ mm^3) was quickly transferred to a solution composed of 80% reservoir solution and 20% glycerol and then cryocooled to 100 K. Data were collected at this temperature on a MAR area detector mounted on an SSRL beam line 9-1. Frames (exposure: 1 min; crystal rotation: 0.5°) were processed using the software programs DENZO[39] and SCALEPACK.[40] The R_{merge} for all data between 30 and 3.0 Å was 7.0% with a 18.8-fold redundancy (Table 6). The crystal unit cell indicated the possible presence of several molecules in the asymmetric unit ($V_M = 4.3$ Å^3/Da of protein for 2, 2.9 Å^3/Da for 3, and 2.15 Å^3/Da for 4 PGK molecules in the asymmetric unit).

Several *T. brucei* PGK structures, which have been determined in our laboratory,[13,14] were used as probes for molecular replacement procedures incorporated in the program AmoRe.[41] The signal-to-noise ratio increased when the partially opened form PGK structure (1.6 Å resolution)[14] was used instead of the closed form PGK structure (2.8 Å resolution).[13] On the basis of this result, a new 'open' form probe was built by superimposing the N- and C-terminal domains of the *T. brucei* PGK structure onto that of horse PGK,[12] which adopts an open conformation. The peaks obtained from this 'open' form probe gave the highest signal-to-noise ratio. The orientations obtained from the rotation function were subsequently used in the translation function. The translation function gave acceptable results for each subunit with reasonable packing when both subunits were placed in the crystal unit cell. After the introduction of two molecules in the asymmetric unit, the correlation coefficient and the *R*-factor were 0.315 and 0.484, respectively.

Refinement was performed with the program X-PLOR[42] and manual rebuilding of the models was performed with the program O.[43] All data between 15.0 and 3.0 Å were used and a bulk solvent correction was applied. Rigid body refinement was performed to position the two molecules more precisely. Subsequently, refinement of the proteins using strict noncrystallographic symmetry was performed. Visual inspection of an electron density map confirmed the presence of the **46b** in the active site and allowed the incorporation of two inhibitors into the model (Figure 2). Further refinement was performed followed by minor modifications of the protein.

The final model consists of 832 protein residues and two **46b** inhibitor molecules. The *R*-factor (R_{free}) dropped to 0.217 (0.282) with acceptable deviations from ideal geometry (Table 6). Analysis of main chain torsion angles using PROCHECK[44] showed that 87% of the residues are located in the most favored regions with 13% occurring in the additional allowed regions of the Ramachandran plot.

Parasite Cultures and Drug Screening Assays. Bloodstream forms of *T. brucei brucei* (strain 427 from K. Stuart, Seattle Biomedical Research Institute, Seattle, WA) were cultured in HMI-9 medium containing 10% fetal bovine serum, penicillin, and streptomycin at 37 °C with 5% CO_2.[45] Bloodstream forms of *T. brucei rhodesiense* (strain STIB900 from S. Croft, London School of Hygiene and Tropical Medicine, London, U.K.) were cultured in HMI-18 medium under the same conditions.[46] Drug sensitivity of the *T. brucei* strain was determined in 96-well microtiter plates with an initial inoculum of 5×10^3 trypomastigotes/well. Drugs were added in serial dilutions for a final volume of 200 μL/well. Parasite growth was quantified after 48 h by the addition of Alamar blue (Alamar Biosciences, Sacramento, CA).[47] Alamar blue quantitation 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 co-culture with mouse 3T3 fibroblasts in RPMI 1640 containing 10% fetal bovine serum, penicillin, and streptomycin at 37 °C with 5% CO_2. Drug sensitivity studies with mammalian stage *T. cruzi* were carried out using the β-galactosidase assay as described.[48] The inhibitory effects of the compounds on growth of mammalian host cells (murine 3T3 fibroblasts) were determined using the Alamar blue method.[48]

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Registry Numbers: 2b, 5059-59-6; **3a**, 14357-08-5; **3b**, 177949-65-4; **4a**, 17270-22-3; **4b**, 26783-33-5; **4c**, 35109-89-8; **5a**, 17270-23-4; **6a**, 23096-10-8; **6b**, 26784-37-2; **7a**, 26293-51-6; **8a**, 126434-89-7; **10b**, 26775-39-3; **10c**, 56720-65-1; **11a**, 35662-05-6; **12a**, 35662-06-7; **13a**, 103842-14-4; **13b**, 103842-19-9; **14a**, 182950-54-5; **16a**, 35662-04-5; **16a′**, 60687-64-1 **16c**, 77112-18-6; **17a**, 34436-52-7; **17a′**, 90275-18-6; **19a**, 60687-66-3; **19a′**, 60687-65-2; **19c**, 77112-19-7; **20a**, 64630-73-5; **20a′**, 64630-69-9; **21a**, 41552-82-3; **21b**, 120524-28-9; **21c**, 57972-89-1; **22a**, 36396-99-3; **22b**, 26783-44-8; **22c**, 53296-12-1; **23a**, 97826-48-7; **24a**, 41552-83-4; **25a**, 36397-01-0; **26a**, 4338-47-0; **26b**, 26783-39-1; **27b**, 26783-32-4; **27c**, 56720-66-2; **28a**, 20315-90-6; **28b**, 26775-40-6; **29a**, 23707-33-7; **29b**, 26783-37-9; **30a**, 35940-03-5; **31a**, 35940-04-6; **32a**, 23707-32-6; **32b**, 26783-35-7; **33a**, 23660-97-1; **34a**, 23660-96-0; **34b**, 26775-37-1; **35a**, 23660-98-2; **35b**, 26775-34-8; **36a**, 23666-24-2; **37a**, 20125-39-7; **37b**, 26775-35-9; **37c**, 56720-67-3; **38a**, 109956-81-2; **38b**, 26783-34-6; **38c**, 124498-68-6; **39a**, 54712-70-8; **39c**, 124498-53-9; **40a**, 101565-57-5; **40c**, 124499-05-4; **41a**, 21924-65-2; **43a**, 98383-40-5; **43b**, 98383-43-8; **44a**, 101565-68-8; **46a**, 41552-94-7; **46b**, 26888-84-4; **46c**, 124499-08-7; **47a**, 54241-03-1; **48a**, 101565-56-3; **49a**, 101565-64-4; **50a**, 101565-65-5; **52a**, 101565-69-9; **53a**, 101565-70-2; **54a**, 101565-71-3; **54c**, 124499-07-6; **55a**, 36799-20-9; **55b**, 26783-40-4; **58a**, 89705-21-5; **58c**, 161536-30-7; **69**, 120524-43-8; **82**, 120524-46-1.

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