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Investigations into the Origin of the Molecular Recognition of Several Adenosine Deaminase Inhibitors

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Inhibitors of adenosine deaminase (ADA, EC 3.5.4.4) are potential therapeutic agents for the treatment of various health disorders. Several highly potent inhibitors were previously identified, yet they exhibit unacceptable toxicities. We performed a SAR study involving a series of C2 or C8 substituted purine-riboside analogues with a view to discover less potent inhibitors with a lesser toxicity. We found that any substitution at C8 position of nebularine resulted in total loss of activity toward calf intestinal ADA. However, several 2-substituted-adenosine, 8-aza-adenosine, and nebularine analogues exhibited inhibitory activity. Specifically, 2-Cl-purine riboside, 8-aza-2-thiohexyl adenosine, 2-thiohexyl adenosine, and 2-MeS-purine riboside were found to be competitive inhibitors of ADA with K_i values of 25, 22, 6, and 3 μM, respectively. We concluded that electronic parameters are not major recognition determinants of ADA but rather steric parameters. A C2 substituent which fits ADA hydrophobic pocket and improves H-bonding with the enzyme makes a good inhibitor. In addition, a *gg* rotamer about C4′−C5′ bond is apparently an important recognition determinant.

Introduction

Adenosine deaminase (ADA[a]) is a major enzyme of purine metabolism, catalyzing the deamination of adenosine, **1a**, and 2′-deoxy-adenosine, **1b**, to the corresponding inosines, **3** (Scheme 1).[1] ADA is ubiquitous in almost all human tissues, and abnormal levels of ADA have been detected in a variety of diseases including AIDS, anemia, lymphomas, and leukemias.[2−6] ADA plays a role not only as a cytosolic enzyme but also as an ecto-enzyme, thus modulating signal transduction through adenosine receptors by regulating extracellular adenosine levels.[7]

The involvement of ADA in various health disorders triggered numerous attempts to develop ADA inhibitors as potential therapeutic agents. Thus, inhibition of ADA can be useful in the therapy of viral infections and of some types of lymphoproliferative disorders.[8−11] Specifically, ADA inhibitors are needed to prevent the deamination of antileukemic and antiviral agents containing adenine bases and their subsequent inactivation.[12−15] In addition, ADA inhibitors can modulate the immune response in B- or T-cell malignancies.[16,17] ADA inhibitors also have great potential as anti-inflammatory agents, as ecto-ADA could induce chronic inflammation by degrading extracellular adenosine, or 2′-deoxyadenosine, which are toxic to lymphocytes.[18] The use of extracellular ADA inhibitors may offer cardiovascular protection in hypertension as well.[19,20]

Although reported more than three decades ago, the most potent ADA inhibitors known are still the natural products coformycin, **4a**, and pentostatin, **4b** (Figure 1), with K_i values of 1×10^{-11} and 2.5×10^{-12} M, respectively.[21,22] Their activity is attributed to the nearly irreversible binding of those compounds with ADA, mimicking the transition state, **2**, of ADA activity (Scheme 1). Synthetic highly potent ADA inhibitors include a pyrazolo pyrimidine analogue, **5**, with K_i value of 53×10^{-12} M,[23] and erythro-9-(2-hydroxy-3-nonyl)adenine, (+)-EHNA, **6**, with K_i in the low nM concentration range (Figure 1).[16,24] Over the past three decades, numerous ADA inhibitors have been developed based on modifications of the nucleoside scaffold, some of them resulting in submicromolar inhibition constants. These modifications include 1-deaza-adenosine, **7** (K_i 10^{-7} M),[25] and 8-aza-nebularine, **8** (K_i 4×10^{-8} M),[26] nebularine, **9** (K_i $7-22 \times 10^{-6}$ M),[26−29] and pyrazolo pyrimidine 4-one, **10** (K_i 1.6×10^{-10} M) (Figure 1).[30] The highly potent inhibitors, such as coformycins and EHNA, proved unsatisfactory for clinical use. Thus, coformycin analogues used for cancer treatment exhibited unacceptable toxicities[31,32] and EHNA derivatives showed poor pharmacokinetics such as rapid metabolism.[33,34] Recently, various attempts to improve the unfavorable properties of the known inhibitors were reported. These approaches include, for instance, the design of modified coformycin analogues that bind less tightly than the parent compounds[35] or of hybridized non-nucleoside ADA inhibitors.[36]

The failure of coformycin analogues in the clinic encouraged us to explore the SAR of the natural substrate, adenosine, **1**, and the related purine-riboside inhibitor, nebularine, **9**, with a view to identify their molecular recognition determinants in ADA. Deciphering the origins of their molecular recognition may help the identification or design of less toxic ADA inhibitors.

For this purpose, we implemented the following strategy: we synthesized adenosine, 8-aza-adenosine, and nebularine analogues substituted at different positions of the purine base,

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[a] Abbreviations: AcCl, acetyl chloride; ADA, adenosine deaminase; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; TMSCl, trimethyl silyl chloride; BTEA-NO_2, benzyl triethyl ammonium nitrite; TMSOTf, trimethyl silyl triflate; TBN, *t*-butyl nitrite; TMSBr, trimethyl silyl bromide; AcCl, acyl chloride; SAR, structure−activity relationship.

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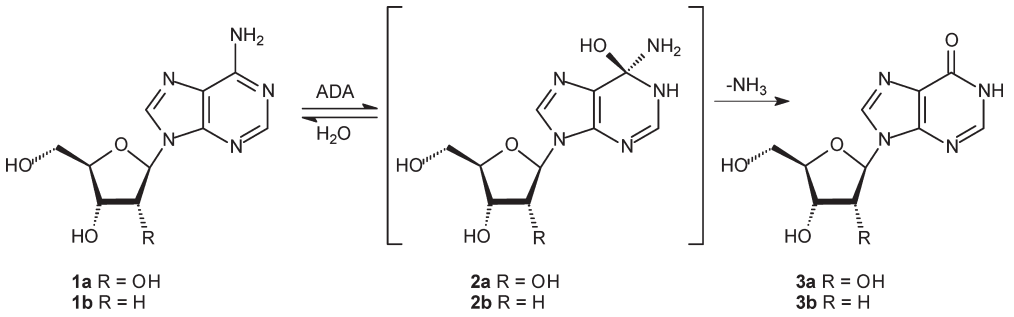
Scheme 1. Adenosine Deamination Reaction Catalyzed by ADA



1a R = OH
1b R = H

2a R = OH
2b R = H

3a R = OH
3b R = H

i.e., analogues, **11**−**25** (Table 1), changing in this way the stereoelectronic properties and conformation of the parent scaffold. These analogues were evaluated as substrates/inhibitors of calf intestine adenosine deaminase. The predominant conformation of these analogues in solution was analyzed by NMR experiments. The electronic properties of the modified scaffold were previously calculated.[37,38] Correlation between K_i values and the electronic and conformational features of these analogues was investigated for the elucidation of the origin of molecular recognition of ADA inhibitors.

Results

Rational Design of Potential ADA Inhibitors. Three dimensional structures of complexes of ADA with various inhibitors have been deciphered by crystallographic,[39−41] computational,[42−47] and NMR studies[48,49] as well as mutagenesis analysis.[50−52] Yet, knowledge about the stereoelectronic and conformational requirements of ADA substrates and inhibitors is still not comprehensive.

Therefore, here we targeted a systematic SAR study of both adenosine substrates and nebularine-based inhibitors with a view to developing improved ADA inhibitors.

The most important interactions of a substrate/inhibitor with ADA are the interactions involving the nucleoside base. These interactions include: H-bond between Gly184 backbone nitrogen and the adenosine N3-nitrogen, and H-bond between Glu217 and the adenosine N1 and N^6 nitrogen atoms, H-bond between Asp296 and the adenosine N7 nitrogen (Figure 2).[23,39] All of these interactions have importance not only in placing the substrate/inhibitor in the right position but also in activating the substrate and stabilizing the reaction intermediate.[50] Thus, we decided to focus on modifications at the adenine or purine moieties of adenosine or nebularine, respectively. The type and the position of the substitution affect the stereoelectronic properties of the purine moiety and the nucleoside conformation and hence affect the molecular recognition of the adenosine/nebularine analogue.

Biagi et al.[53] proposed based on SAR study on EHNA, **6**, derivatives that there is a hydrophobic pocket near the adenine C2, namely a bulky substitution at this position may have a significant interaction with this pocket and consequently enhance affinity to ADA. To test this hypothesis, we designed a series of 2-substituted-adenosine, 8-aza-adenosine, and nebularine derivatives.

To test the tolerance of the ADA hydrophobic pocket near the adenosine C2 position, we prepared 2-*S*-alkyl adenosine analogues with increasing alkyl length and bulkiness (e.g., from SMe, **22**, to *S*-benzyl, **25**).

Likewise, to evaluate the electronic effects in 2-substituted nebularine analogues, we prepared 2-substituted nebularine with both electron withdrawing group (2-Cl-nebularine, **11**) and electron donating groups (**12**−**14**), and to evaluate the role of steric effects on the recognition of 2-susbtituted nebularine analogues, we compared the activity of 2-NHMe-nebularine, **13** to that of 2-$NHCH_2$Ph-nebularine, **14**.

It has been reported that although ADA has a lower binding affinity toward 8-aza-adenosine as compared to adenosine, this derivative is deaminated much faster than adenosine itself.[54] Thus, to test whether 8-aza-adenosine analogues are more potent substrates than the respective adenosine analogues, we prepared 2-*S*-alkyl(benzyl) 8-aza-adenosine analogues, **20**−**21**, and compared their activity to the respective 2-*S*-alkyl(benzyl) adenosine analogues, **22**−**25**.

To evaluate the role of the adenine N^6 amine in molecular recognition, we compared the activity of 2-SMe-adenosine, **22**, and the corresponding nebularine analogue **12**.

To evaluate the significance of the substituent position on the molecular recognition by ADA, we prepared 2-SMe-nebularine and 8-SMe-nebularine, **12** and **18**.

C8-substituted adenosine analogues bearing nonbulky substituents are moderate to poor ADA inhibitors with K_i values in the range of 22−123 μM.[54] Therefore, for comparison, we prepared C8-substituted nebularine analogues, **15**−**19**, bearing a small electron donating substituent at C8, such as OMe, NHMe, SMe, NH_2, and SH group.

Synthesis. A series of C2-substituted adenosine, 8-aza-adenosine, and nebularine has been synthesized (Schemes 2−7). 2-Amino peracylated-nebularine,[55] **26**, was used as a common synthetic intermediate for the preparation of 2-Cl,[56] 2-SMe,[56] 2-NHMe, and 2-$NHCH_2$Ph-nebularine[57] analogues, **11**−**14** (Scheme 2). 2-Cl-peracylated-nebularine,[56] **27**, was obtained in 86% yield from **26** by treating the latter with TMSCl and benzyltriethylammonium nitrite (BTEA-NO_2) at room temperature overnight.[58] These conditions were superior to regular chloro-dediazotation conditions.[59] Subsequently, the acetate protecting groups were removed in methanolic ammonia to provide **11** in 89% yield, which was superior to conditions reported in the literature.[56] 2-Methylthio-nebularine, **12**, was obtained in 56% yield upon treatment of **27** with sodium methyl mercaptide in DMF and H_2O at room temperature overnight[49] and subsequent treatment with ammonium hydroxide in ethanol/dioxane solution at 60 °C for 4 h, these conditions were superior to sodium methyl mercaptide in methanol reported previously[56] as under these conditions both 2-methylthio- and 2-methoxy-purine riboside were obtained in a mixture that was impossible to separate. 2-Methylamino-nebularine, **13**, was obtained in 38% yield from **27** by treatment with solution of NH_2Me in MeOH at 70 °C for 3 h in a sealed tube. These conditions result in both C2 substitution by the NHMe group and complete deprotection of the ribose hydroxyl groups. Finally, 2-$NHCH_2$Ph-nebularine,

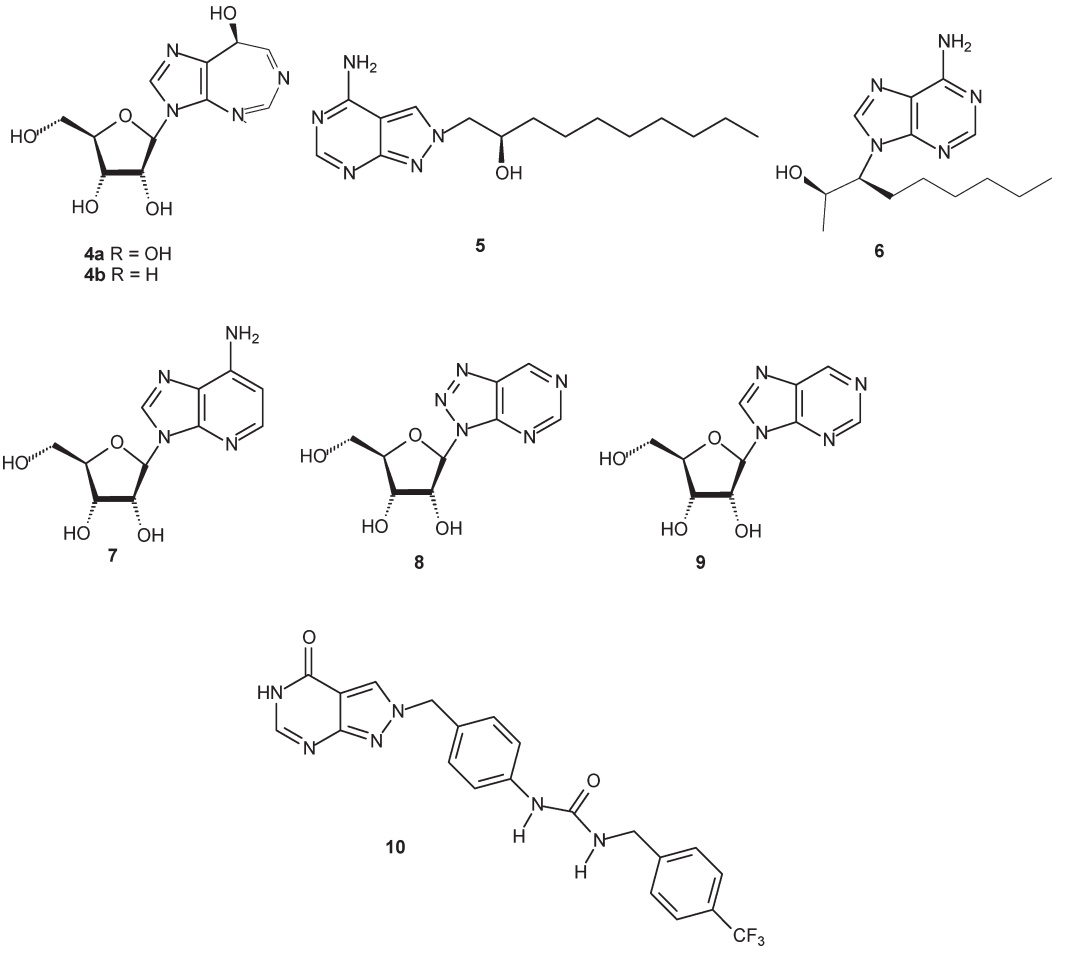
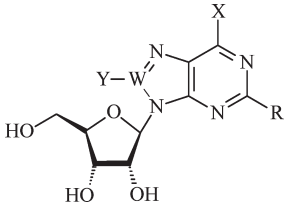


Figure 1. Several known ADA inhibitors.

Table 1. Adenosine, 8-Aza-adenosine, and Nebularine Analogues Synthesized and Evaluated in This Study



	X	W	Y	R
11[56]	H	C	H	Cl
12[56]	H	C	H	SMe
13	H	C	H	NHMe
14[57]	H	C	H	$NHCH_2Ph$
15[60]	H	C	OMe	H
16[60]	H	C	NH_2	H
17	H	C	NHMe	H
18[60]	H	C	SMe	H
19[60]	H	C	SH	H
20	NH_2	N		$SCH_2C_6H_5$
21	NH_2	N		$S(CH_2)_5CH_3$
22[75]	NH_2	C	H	SMe
23[75]	NH_2	C	H	$S(CH_2)_3CH_3$
24[75]	NH_2	C	H	$S(CH_2)_5CH_3$
25[75]	NH_2	C	H	$SCH_2C_6H_5$

reported recently in the literature,[57] **14**, was obtained in 20% yield upon treatment of **27**, with benzylamine in DMF at 60 °C.

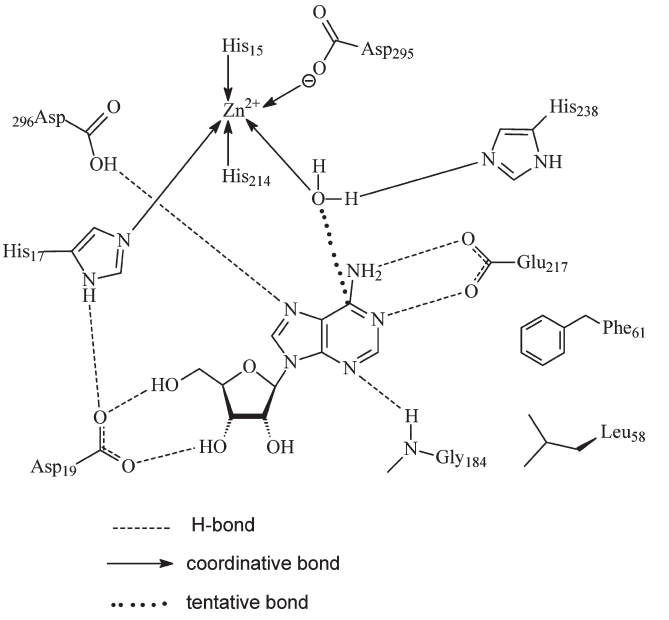
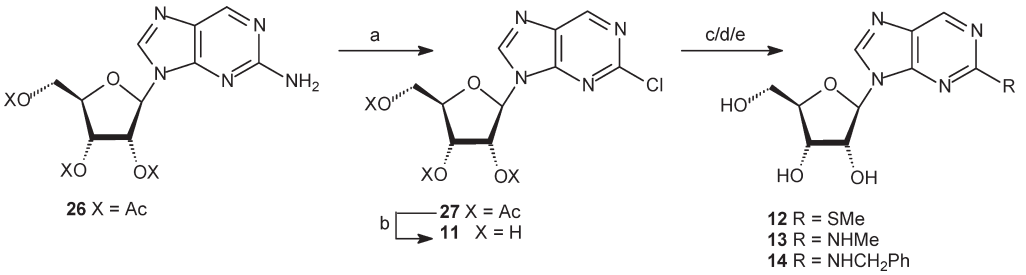


Figure 2. Hydrogen bond network of adenosine in ADA active-site adopted from ref 23.

The low yield is due to decomposition of the starting material under these conditions.

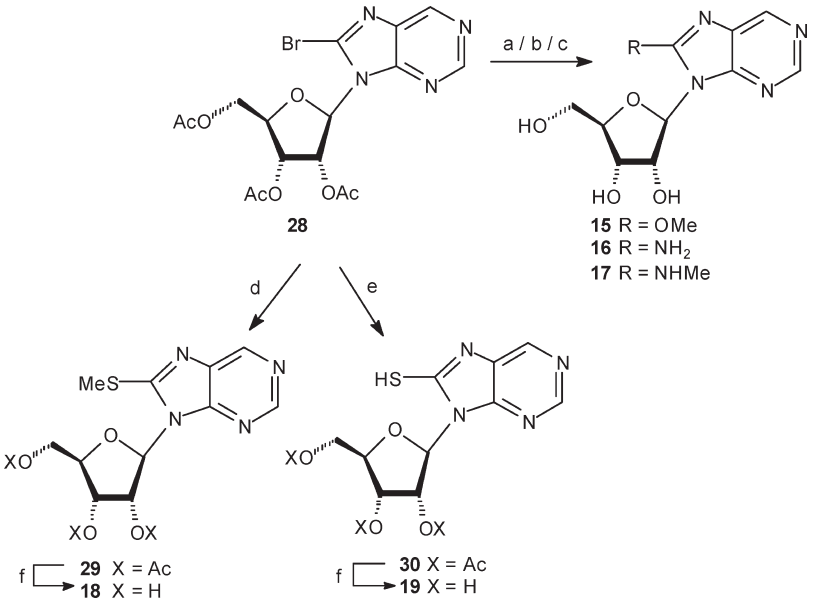
2′,3′,5′-Tri-*O*-acetyl-8-bromonebularine, **28**,[60] was used as a common starting material for the preparation of all

Scheme 2. Synthesis of 2-Substituted Nebularine Analogues[a]



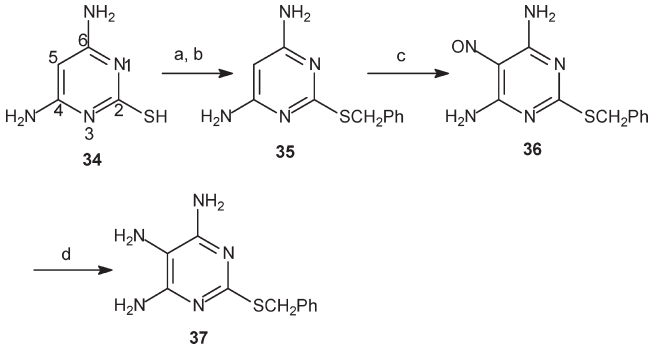
[a] Reagents and conditions: (a) BTEA-NO_2, TMSCl, CH_2Cl_2, RT, 16 h, 86%; (b) NH_3/EtOH, 4 °C, 16 h, 30%; (c) NaSMe, MeOH, 60 °C, 4 h, 56%; (d) NH_2Me/EtOH, 70 °C, 3 h, 38%; (e) NH_2CH_2Ph/DMF, 100 °C, 24 h, 20%.

Scheme 3. Synthesis of 8-Substituted Nebularine Analogues[a]



[a] Reagents and conditions: (a) NaOMe/MeOH, 4 °C, 16 h, 76%;[60] (b) NH_2Me/EtOH, 0 °C, 2.5 h, 24%; (c) NH_3/EtOH, RT, 16 h, 37%; (d) NaSMe/DMSO, RT, 2 h;[60] (e) thiourea/EtOH, RT, 2 h;[60] (f)1 M NaOH, MeCN/H_2O, RT, 1 h.[60]

Scheme 4. Synthesis of Pyrimidine Analogue **37**[a]



[a] Reagents and conditions: (a) NaOH/MeOH, RT, 1 h; (b) BzBr/DMF, rt, 16 h, 89%; (c) $NaNO_2$, AcOH, 0 °C, 4 h, 89%; (d) H_2/EtOH, PtO_2, RT, 4 h, 100%.

C8-substituted nebularine analogues (Scheme 3). Compound **15** was obtained upon treatment of **28** with sodium methoxide as reported previously.[60] Compound **16** was obtained upon treatment of **28** with methanolic ammonia, this procedure was found to be superior to that reported in the literature because the product was obtained in one step rather than three steps in the previous report.[60]

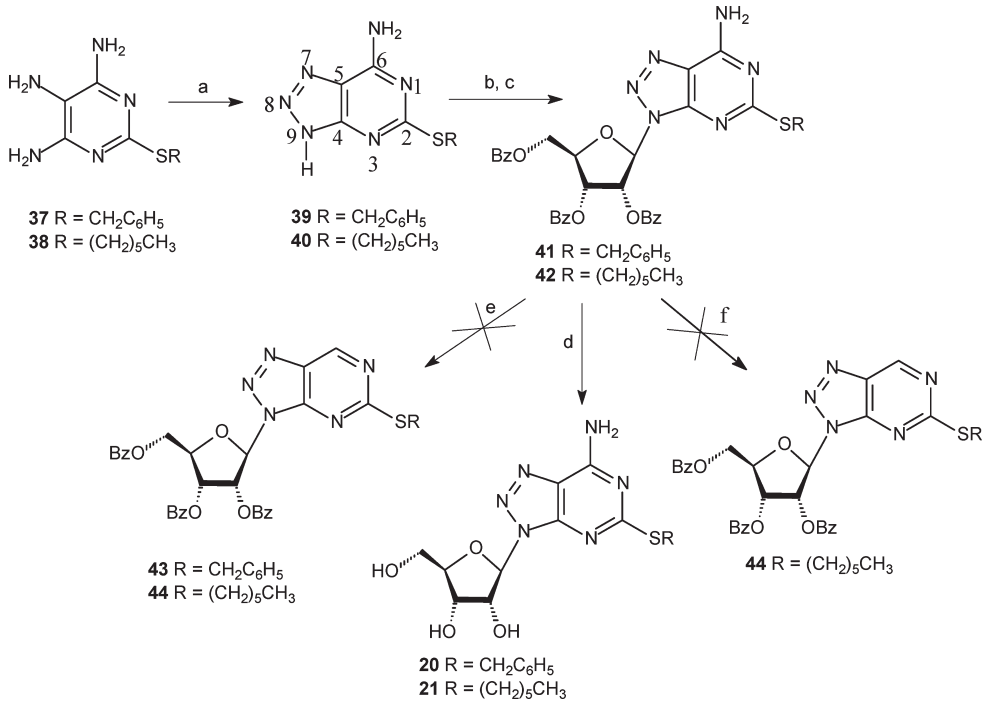
2-NHMe-nebularine, **17**, was prepared by treatment of **28** with ethanolic methylamine in a sealed tube at 0 °C. In addition to the C8-substitution, full deprotection of the sugar hydroxyl groups was achieved under these conditions.

8-SH- and 8-MeS-nebularine, **18**−**19**, were obtained as reported in the literature.[60]

For the synthesis of 2-thioalkyl-8-aza-adenosine analogues, we attempted the preparation of a common building block, 2-mercapto-8-aza-adenine, **32**, which was supposed to be *S*-alkylated by various alkylating agents and then ribosylated to yield the desired products. For this purpose, 4,5,6-triamino-2-mercapto-pyrimidine, **31**, prepared as described before,[61] was treated with sodium nitrite/HCl.[58] The cyclization of **31** to 8-aza-adenine involves attack of the amine group in **31** on the NO^+ formed from sodium nitrite and HCl, followed by attack of the second amine group on the aza-hydroxy intermediate.

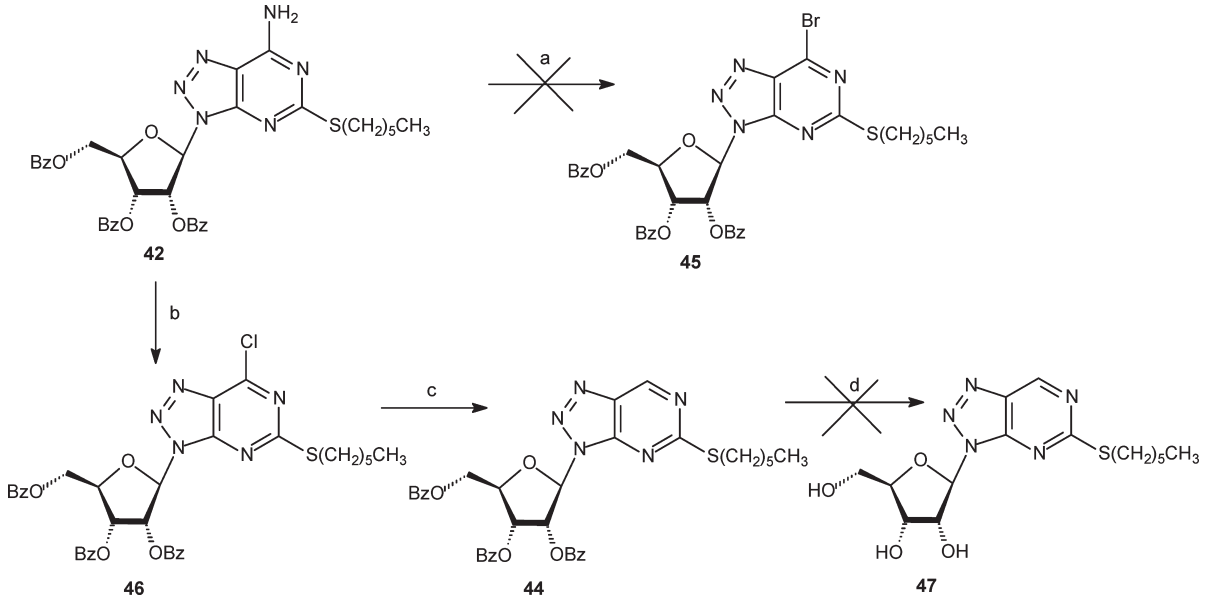
Yet, although the ring cyclization did occur, the desired product **32** was not obtained but, rather, a disulfide dimer of 2-mercapto-adenine, **33**, as indicated by TOF MS ES+ (MH^+ 335). Apparently, HONO formed in the reaction has oxidized the thiol function to disulfide.[62] Attempts to reduce disulfide, **33**, back to **32** with mercaptoethanol at room temperature were not successful, and heating **33** to 60 °C resulted in its decomposition.

Scheme 5. Synthesis of 8-Aza-nebularine and Adenosine Analogues[a]



[a] Reagents and conditions: (a). $NaNO_2$, HCl, 0 °C, 4 h, 73% for **40**, 55% for **39**; (b) HMDS, $(NH_4)_2SO_4$, 140 °C, 1 h; (c) TMSOTf; 1,2-DCE; 1′-acetate, 2′,3′,5′-tribenzoate-β-D-ribose, 1,2-DCE, 120 °C, 3 h, 23% for **39**, 20% for **40**; (d) NH_4OH; dioxane; MeOH, 23% for **20**, 71% for **21**; (e) isoamylnitrite; THF, 100 °C, 4 d; (f) $NaNO_2$, Cl_2CHCO_2H/THF, 6 h, RT.

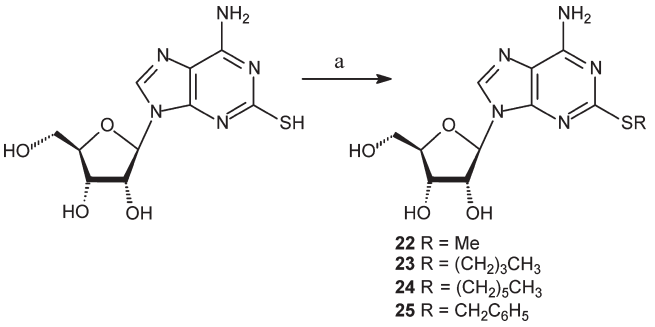
Scheme 6. Attempted Synthesis of Analogue **47**[a]



[a] Reagents and conditions: (a) TBN/TMSBr, CH_2Br_2, 6 h, 0 °C; (b) AcCl/BTEA-NO_2, CH_2Cl_2, 4 h, 0 °C, 60%; (c) Pd/C, EtOH, NaOAc, 7 h, RT, 50%; (d) 2 M NH_3/EtOH, 70 °C, 24 h, or NH_4OH/dioxane/MeOH.

Therefore, we decided to apply an alternative synthetic approach which includes first alkylation of the thiol function in **32**, to prevent oxidation, and then introduction of the nitrogen and ring cyclization to the 8-aza-adenine (Schemes 4, 5). Specifically, 2-benzylthio-8-aza-adenosine was constructed from pyrimidine **37**. The latter pyrimidine analogue was prepared from pyrimidine **34** as follows: (1) Selective S-benzylation of analogue **34** was achieved upon treatment of **34** with NaOH in a methanolic solution at room temperature to form the corresponding sodium thiolate salt. The latter was freeze-dried and added to benzyl bromide in DMF. Product **35** was obtained in 89% yield. (2) Nitrosation of **35** was achieved with $NaNO_2$ in aqueous acetic acid[63] in 86% yield, as indicated by the disappearance of H-5 in ^{1}H NMR and appearance of four different aromatic carbons (177.1 (C-2), 166.0 (C-5), 148.6 (C-4), 139.7 (C-6) ppm). The resulting blue nitroso compound **36** was reduced in a quantitative yield by catalytic hydrogenation over PtO_2 at atmospheric pressure

Scheme 7. Synthesis of 2-Substituted Adenosine Analogues[a]



[a] Reagents and conditions: (a) 0.25 M NaOH, freeze-drying then addition of RBr, DMF, RT, 24 h.[75,90]

to produce **37**. The production of the latter compound was verified by TOF MS ES+ analysis (MH^+ 248) and the shift of the C-5 chemical shift (from 166 in **36** to 102 ppm in **37**).

The triamino-pyrimidine 2-thiohexyl analogue **38** was prepared as we described before.[61] Treatment of **37** or **38** with sodium nitrite/HCl[64] resulted in the precipitation of a green solid, which upon crystallization afforded 2-hexylthio-8-aza-adenine, **40**, and 2-benzylthio-8-aza-adenine, **39**, both as a yellow solid in 72% and 55% yield, respectively (Scheme 5). The structure of **40** was elucidated by UV (λ_{max} 246 nm instead of 280 nm for the triamino analogue) and TOF MS ES+ (MH^+ 253).

Ribosylation of **39** and **40** to obtain the desired 8-aza-adenosine analogue posed a severe regio- and stereoselectivity problem as coupling of **39**/**40** with a protected ribose may give rise to several products. Coupling reaction can occur at either N7/N9 or N8 positions.[65,66] Furthermore, the reaction is not stereospecific and can occur either from the α or β face of the ribose ring. Different ribosylation conditions such as solvent, reaction temperature, catalyst, and sugar protective groups determine the formed products and their distribution.

First, we optimized the ribosylation reaction conditions using analogue **40**. The sugar moiety selected for the coupling was 1′-acetate, 2′,3′,5′-tribenzoate-β-D-ribose. Treatment of this sugar with Lewis acid, such as trimethyl silyl triflate (TMSOTf) or $SnCl_4$ produces electrophilic sugar cation anchimerically stabilized by the neighboring benzoyl group.[67] The stabilized sugar-cation controls the β-selectivity of the product.[68]

Additional parameters influence the selectivity of the ribosylation reaction: conditions that favor kinetic control lead to formation of N7/N8 isomers, whereas conditions that favor thermodynamic control lead to formation of N9 isomer. The N8-ribosylated-8-aza-adenine was proposed to be the stable product of 8-aza-adenine ribosylation compared to the N7-ribosylated product.[65]

Thus, reaction of 1′-acetate, 2′,3′,5′-tribenzoate-β-D-ribose with **40** catalyzed by $SnCl_4$ at room temperature (kinetic control) did not produce the N9 isomer, **42**, at all. Prior silylation of **40** with HMDS/$(NH_4)_2SO_4$ in refluxing dichloroethane and then reaction with 1′-acetate, 2′,3′,5′-tribenzoate-β-D-ribose in the presence of $SnCl_4$ catalyst at room temperature afforded the N8-isomer (β-D-anomer) that was identified by NOE spectrum.[69]

Finally, nucleoside **42** (N9 isomer) was obtained from **40** upon ribosylation under the Vörbruggen conditions[68] (thermodynamic control).[70] Specifically, 1′-acetate, 2′,3′,5′-tribenzoate-β-D-ribose was added to the silylated heterocycle

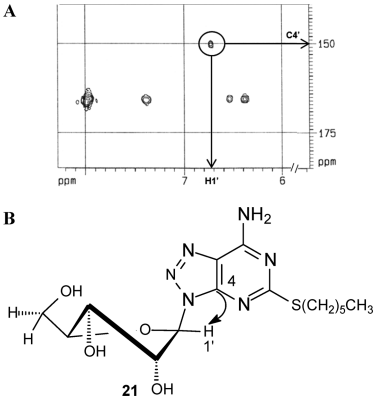


Figure 3. (A) HMBC spectrum of 8-aza-2-thiohexyl-adenosine, **21**. (B) Conformation of **21** based on HMBC spectrum.

40 (HMDS/$(NH_4)_2SO_4$) in the presence of TMSOTf in dichloroethane under reflux for 4 h. This method provided the desired product in ca. 20% yield. Yet when the initial quantities of the reactants were scaled up from 0.4 to 14 mmol, the yield increased up to 60%. Finally, product **42** was deprotected by NH_4OH in MeOH−dioxane mixture to provide product **21** in 71% yield. The regio- and stereochemistry of the product was characterized by NMR spectra. Specifically, HMBC spectrum helped identify whether the product was the N8 or N9-isomer by observing the crosspeak between the spatially adjacent C4 and H1′, which is only possible if the isomer is N9 (Figure 3). The β-configuration was confirmed by comparing coupling constants $^3J_{H1'-H2'}$ of **21** to those of adenosine.

Applying the same ribosylation conditions to **39** yielded the product **41** in 23% yield. Attempts to prepare 8-aza-nebularines **43** and **44** from **41**, **42**, respectively, by a common deamination procedure[71] involving isoamylnitrite/THF failed and nucleosides **41** and **42** remained intact. Furthermore, deamination by sodium nitrite in the presence of dichloroacetic acid[72] did not result in deaminated product either.

Another strategy to obtain **44** was implemented, which involves deamination in two steps (Scheme 6) by first changing 6-amino group to a better leaving group (bromo or chloro) and then dehalogenation.

First, we attempted the preparation of 6-bromo-8-aza-2-HexS-adenosine-2′,3′,5′-tribenzoate, **45**. A common reagent for the deamination-halogenation reaction is nitrosyl halide (NOCl or NOBr) formed in situ.[73,74] We selected *tert*-butyl nitrite (TBN) and trimethyl silyl bromide (TMSBr)[58] as a combination of reagents for the generation of NOBr in situ, as these reagents are mild and applicable to substituted adenosine. However, reaction of **42** with TBN/TMSBr in dibromomethane for 6 h at 0 °C did not afford the desired product as indicated by both ^{13}C NMR and MS.

Then, we attempted to prepare 6-chloro-8-aza-2-HexS-adenosine-2′,3′,5′-tribenzoate, **46**. Thus, treatment of 8-aza-2-HexS-adenosine-2′,3′,5′-tribenzoate, **42**, with a 5-fold excess of AcCl/BTEA-NO_2 in CH_2Cl_2 at 0−5 °C for 4 h,[58] gave the desired product in 60% yield.

Dehalogenation was achieved by hydrogenation over Pd/C in ethanol as solvent. Dry sodium acetate was added to neutralize the HCl, formed during the reaction, thus providing product **44**, in 50% yield.

Yet, deprotection of the 2′,3′,5′-hydroxyl groups was not feasible. When product **44** was treated with 2 M ammonia in

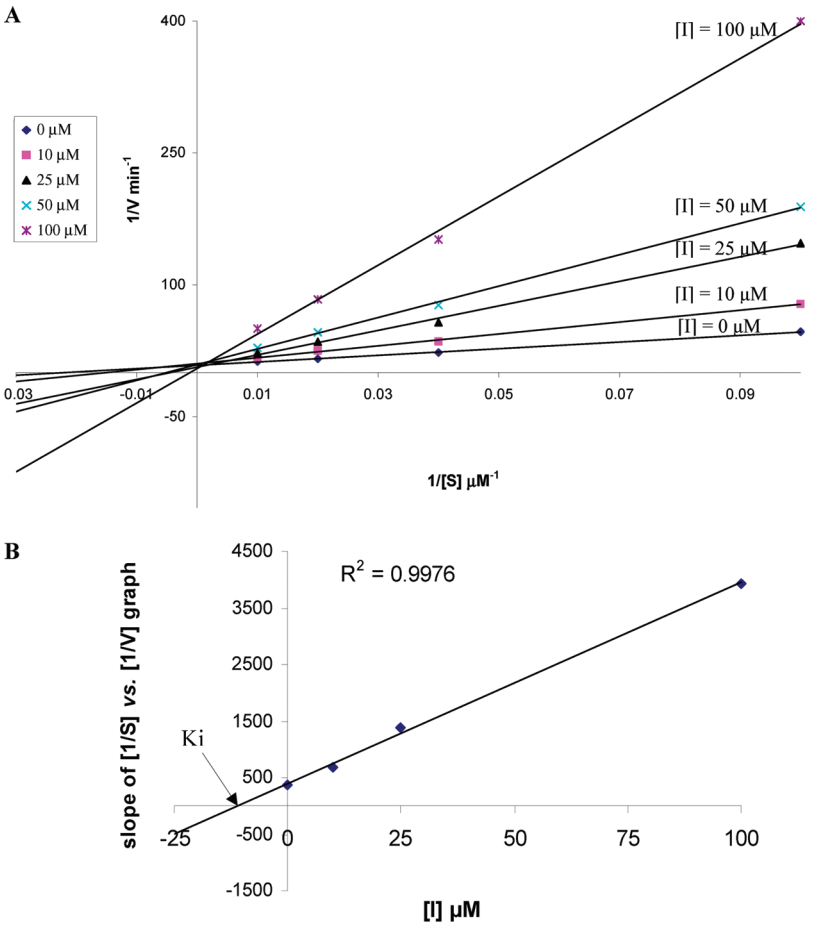


Figure 4. (A) Lineweaver−Burk plot for 8-aza-2-HexS-adenosine, **21**, showing competitive inhibition of ADA. (B) Plot of slope of [1/*S*] vs 1/*V*, vs [I] for analogue **21**.

ethanol for 24 h at 70 °C, **44** remained unchanged. However, using more severe conditions: ammonium hydroxide in dioxane and methanol at reflux in a sealed tube resulted in decomposition of the starting material.

2-Thioalkyladenosine analogues **22**−**25**, were prepared from adenosine as we described before (Scheme 7).[75] 8-Thiomethyl adenosine, **48**, was prepared according to our previously reported procedure.[75]

Evaluation of Analogues 11−**25 as ADA Substrates/Inhibitors.** Analogues **11**−**25** were evaluated both as substrates and inhibitors of calf intestine ADA.[53] First, adenosine analogues were evaluated as substrates. For, this purpose, the UV spectrum of each analogue, **20**−**25**, was measured and λ_{abs} was determined. Then, the hydrolysis of those adenosine analogues in the presence of ADA was monitored by the decrease of the analogue absorbance at the corresponding λ_{abs}. None of analogues **20**−**25** proved to be a substrate of ADA.

The inhibitory activity of adenosine and nebularine analogues, **11**−**25**, at 10−100 μM concentration was determined in the presence of 25−100 μM adenosine. The rate of hydrolysis of adenosine to inosine was determined by monitoring spectrophotometrically the decrease in absorbance at 265 nm over 2 min. Specifically, five Lineweaver−Burk graphs (1/*V* vs 1/[*S*]) were plotted for each inhibitor, each graph representing a different inhibitor concentration. Lineweaver−Burk graphs slope vs inhibitor concentration [*I*] were plotted (adenosine and inhibitors concentration range was 10−100 μM and 20−100 μM, respectively), and K_i was determined by the intercept with the *X*-axis (e.g., Figure 4 for compound **20**). Each assay was repeated 2−5 times on different days.

We compared our K_i values to those reported in the literature (Table 2). 2-Alkylthio-substituted adenosine analogues **22**−**25** are more potent than 2-alkylamino-substituted adenosine analogues and 2-Cl-adenosine.[76] 8-Aza-adenosine analogues substituted at C2, **20**−**21**, are more potent than 8-aza-adenosine itself.[51] 2-Substituted-nebularine analogues, **11**−**14**, are potent inhibitors of ADA. 2-MeS-nebularine, **12**, is about equipotent as 2-amino-nebularine[21] yet more potent than nebularine itself.[26−29] 8-Substituted nebularine analogues, **15**−**19**, proved poor inhibitors of ADA (K_i > 100 μM) as compared to 8-substituted adenosine analogues which are moderately potent inhibitors with K_i values in the range of 22−123 μM.[54,76]

To elucidate the molecular recognition determinants of analogues **11**−**25**, we next attempted to correlate their K_i values with their conformation. For this purpose, we analyzed the preferred conformation of several 8- or 2-substituted nebularine and adenosine analogues in solution by ^{1}H NMR, ^{13}C NMR, and NOESY experiments.

Conformational Analysis of 8- vs 2-Substituted Nebularine and 8-Azaadenosine Analogues. The nucleoside analogues selected for this study did not dissolve in D_2O, thus the solvent of choice was CD_3OD. This solvent not only dissolves all analogues but also retains hydrogen bonding similar to water. The chemical shifts and splitting patterns of purine and sugar protons of 8-MeS-purine riboside, **18**, 8-MeS-adenosine, **48**, 2-MeS-purine riboside, **12**, and

2-HexS-8-aza-adenosine, **21**, were assigned and analyzed as described below (Table 3).

Conformation around the Glycosidic Bond. The quantitative determination of the conformation around the glycosidic bond, χ angle can be obtained by monitoring the vicinal coupling constants $^3J_{C8-H1'}$ and $^3J_{C4-H1'}$, which are extracted from decoupled ^{13}C NMR spectra. A rule for determining whether the base is *anti* or *syn* relative to the sugar was formulated: if a value of $^3J_{C4-H1'} < {}^3J_{C8-H1'}$, the conformation is *anti*, whereas the reverse indicates *syn* conformation. However, this method is only suitable for 8-MeS-nebularine, **18**, 2-MeS-nebularine, **12**, and not for 2-HexS-8-aza-adenosine, **21**, as it has N8 instead of C8 atom. Thus, the preferred conformation of 8-MeS- and 2-MeS nebularine is *syn* and *anti*, respectively (Table 3 for $^3J_{C4-H1'}$ and $^3J_{C8-H1'}$ values). The value of χ angle can be calculated from the following equations which are reparametrized Karplus equations:[77]

$$^3J_{C8-H1'} = 4.5\cos^2(\chi - 60^\circ) - 0.6\cos(\chi - 60^\circ) + 0.1$$

$$^3J_{C4-H1'} = 4.7\cos^2(\chi - 60^\circ) - 2.3\cos(\chi - 60^\circ) + 0.1$$

The second equation provides value of χ in the *high-anti* range for 2-HexS-8-aza-adenosine, **21**, but we can not exclude the possibility that this nucleoside is in equilibrium between *syn* and *anti*. This is consistent with data for 8-aza-adenosine reported to be more flexible than adenosine around the glycosidic bond.[78] Thus, the rotational barrier for the glycosidic bond in 8-aza-adenosine is about 0.5 kcal mol^{-1} compared to 6 kcal mol^{-1} for adenosine.[78]

Sugar Puckering. The conformation of the D-ribose ring of nucleoside analogues **12**, **18**, **21**, and **48** was analyzed in terms of equilibrium between two favored puckered conformations: *N* conformer and *S* conformer.[79,80] Populations of each conformation is calculated from $J_{1'2'}$ and $J_{3'4'}$ couplings.[81] Relative portion of each conformer can be calculated from the following equations:

$$J_{1'2'} = 9.3(1 - X_N) = 9.3X_S \quad (1)$$

$$J_{2'3'} = 4.6X_N + 5.3(1 - X_N) \quad (2)$$

$$J_{3'4'} = 9.3X_N \quad (3)$$

The equilibrium constant K_{eq} can be calculated directly from the observed $J_{1'2'}$ and $J_{3'4'}$ values:

$$K_{eq} = X_S/X_N = J_{1'2'}/J_{3'4'} \quad (4)$$

Using the assigned *J*-coupling constants (Table 3), the mole fraction of *S* and *N* conformers were calculated and the results are summarized in Table 4. For 8-MeS-purine riboside, **18**, and 2-MeS-nebularine the predominant sugar puckering is *S* (69 and 58% respectively), while for 2-HexS-8-aza-adenosine, **21**, there is a minor preference to the *N* conformer (52% *N*).

Conformation of Exocyclic CH_2OH Group. There are three classical staggered rotamers with a preferred gauche−gauche (*gg*) conformation for torsion angle γ (torsion angle around the C4′−C5′ bond) in nucleosides.[80] The mole fraction for each staggered rotamer of C4′−C5′ can be calculated from the following equations:

$$\rho_{gg} = [(J_t + J_g) - (J_{4'5'} + J_{4'5''})]/(J_t - J_g) \quad (5)$$

$$\rho_{tg} = (J_{4'5'} - J_g)/(J_t - J_g) \quad (6)$$

$$\rho_{gt} = (J_{4'5''} - J_g)/(J_t - J_g) \quad (7)$$

The coupling constants for pure rotamers were estimated as $J_g = 2.04$ Hz and $J_t = 11.72$ Hz from the Karplus

Table 2. Comparison of K_i Values of Newly Synthesized and Known Inhibitors

compd no.	compd name	K_i (μM)
22	2-MeS-adenosine	33.00
23	2-BuS-adenosine	6.60
24	2-HexS-adenosine	5.90
25	2-BnS-adenosine	6.00
21	2-HexS-8-aza-adenosine	10.00
20	2- BnS-8-aza-adenosine	45.00
	2-MeNH-adenosine	33.00[76b]
	2-EtNH-adenosine	29.80[76b]
	2-nPrNH-adenosine	24.30[76b]
	2-nPrS-adenosine	11.40[76b]
	2-Cl-adenosine	54.50[76b]
48	8-MeS-adenosine	32.10[76b]
	8-aza-nebularine	0.04[26]
18	8-MeS-nebularine	> 100 [a]
15	8-MeO-nebularine	> 100 [a]
17	8-MeNH-nebularine	> 100 [a]
16	8-NH_2-nebularine	> 100[a]
19	8-SH-nebularine	> 100[a]
	8-EtS-adenosine	22.50[76b]
	8-NH_2-adenosine	89.50[76b]
	8-OH-adenosine	123.00[76b]
	nebularine	7.00,[28] 15.30,[29] 16.00,[26] 22.00[27]
11	2-Cl-nebularine	37.40
12	2-MeS-nebularine	3.00
13	2-MeNH-nebularine	76.00
14	2-BnNH-nebularine	46.00
	2-NH_2-nebularine	4.20[83]

[a] nd: not determined (K_i > 100 μM). [b] Compounds in ref 76 were tested at placental ADA.

Table 3. ^3J-Coupling Constants (Hz) of Selected 2- and 8-Substituted Nebularine Analogues, **12**, **18**, **21**, and **48**

compd	1′2′	2′3′	3′4′	4′5′	4′5″	C8, H1′	C4, H1′
18	6.6	5.4	3	3.6	4.2	3.0	5.5
12	5.4	7.0	3.8	3.3	3.8	5.0	2.0
21	4.5	4.8	4.8	3.6	4.8		2.3
48	6.6	5.4	3.0	2.4	3.0		4.5

Table 4. Conformational Analysis of Selected 2- and 8-Substituted Purine Riboside Analogues, **12**, **18**, **21**, and **48** in CD_3OD

	sugar puckering				C4′−C5′ bond			
glycosidic bond								
(**18**) *syn*	(**18**) %	(**12**) %	(**21**) %	(**48**) %	(**18**) %	(**12**) %	(**21**) %	(**48**) %
(**12**) *anti*	$X_S = 69$	$X_S = 58$	$X_S = 48$	$X_S = 71$	*gg* = 21	*gg* = 69	*gg* = 62	*gg* = 86
(**21**) *high-anti*	$X_N = 31$	$X_N = 42$	$X_N = 52$	$X_N = 29$	*tg* = 16	*tg* = 13	*tg* = 16	*tg* = 4
(**48**) *syn*	$K_{eq} = 2.27$	$K_{eq} = 1.38$	$K_{eq} = 0.94$	$K_{eq} = 2.44$	*gt* = 63	*gt* = 18	*gt* = 22	*gt* = 10

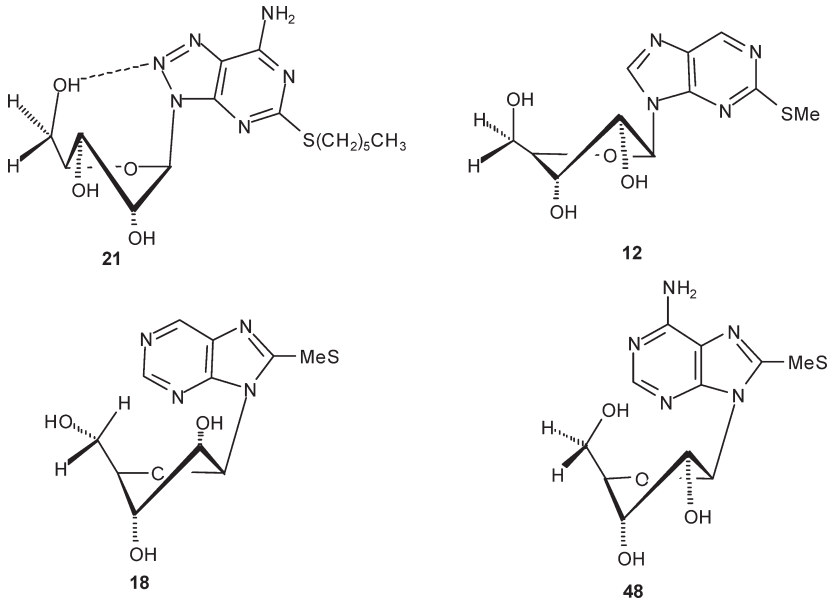


Figure 5. Preferred conformations of analogues **12**, **18**, **21**, and **48** in solution as concluded from NMR data.

equation.[80,82] The populations of *gg*, *tg*, and *gt* rotamers around C4′−C5′ bond were calculated for **12**, **18**, **21**, and **48** as presented in Table 4. We noticed a marked preference for a *gg* rotamer about C4′−C5′ bond for 8-MeS-adenosine, **48**, (86%), 2-MeS-purine riboside, **12**, (69%), and 2-HexS-8-aza-adenosine, **21**, (62%), while for 8-MeS-adenosine, **18**, the preferred conformation is *gt* (63%) (Figure 5).

Discussion

The above SAR study provides comprehensive understanding of ADA binding requirements. Thus, by varying electronic and steric parameters in adenosine, 8-aza-adenosine, and nebularine scaffolds, and analyzing their effect on K_i and nucleoside conformation, we have been able to establish important structure−activity relationships. In addition to stereoelectronic effects, the conformation of nucleosides is an important factor in enzyme binding.[83] Specifically, a nucleoside substituent affects favored ribose ring conformation and rotamer distribution in nucleosides. ADA recognizes preferentially nucleosides in the *anti* conformation having *N* ribose puckering.[84]

The dependence of K_i values of the investigated compounds, **11**−**25**, on stereoelectronic and conformational factors is discussed below for each series of nucleoside analogues.

C8-Substituted Nebularine Analogues. C8 substituted nebularine derivatives, **15**−**19**, were poor inhibitors of ADA ($K_i > 100\ \mu$M). The inactivity of these derivatives is explained by their *syn* conformation (e.g., **18**, Figure 5) as well as their clear preference for ribose *S* conformation (Table 4), whereas ADA recognizes preferentially nucleosides in the *anti* conformation and *N* ribose puckering.[84] Furthermore, the torsional angle about C4′-C5′ is predominantly *gt* (Table 4) unlike most nucleosides which prefer the *gg* rotamer (Figure 5). In the related 2-SMe-adenosine, **48**, the preferred rotamer is *gg*, possibly because of increased electron density on N1/N3, due to electron donation by N^6-amine, resulting in H-bond of 5′-OH with N1/N3 and 86% *gg* population. The lack of N^6-amine in **18** reduces *gg* population to 21%. Therefore, we assume that *gg* rotamer about C4′−C5′ bond may be another conformational requirement for binding to ADA.

C2-Substituted-Adenosine and 8-Aza-adenosine Analogues. Adenosine and 8-aza-adenosine analogues substituted at the C2 position, **20**−**25**, proved nonsubstrates of ADA. Despite the presence of an exocyclic amine at C6, these analogues are not hydrolyzed, possibly due to the shift of C6 from the essential water molecule responsible for the hydrolysis. These analogues, however, proved efficient ADA inhibitors with K_i values ranging from 5.9 to 45 μM (Table 2). These analogues inhibit the deamination of adenosine because they bind tighter to the enzyme than adenosine, possibly due to both conformational compatibility and additional hydrophobic interactions. Specifically, analogues **20**−**25** adopt *anti* conformation (e.g., **21**, Figure 5), which is the preferred conformation by ADA, and N/S populations are practically equal.[77] In addition, the interaction of C2-alkyl/benzyl substitution with a hydrophobic pocket of ADA located near the adenosine C2 position possibly results in a tighter fit.

2-SBu-, 2-SHex-, and 2-SBn-adenosine analogues, **23**−**25**, proved to be moderately good ADA inhibitors (K_i ca. 6 μM) compared with 2-SMe-adenosine, **22**, (K_i 33 μM). The difference between **22** and **23**−**25** is possibly due to a tighter fit of the latter compounds with ADA's hydrophobic pocket. This pocket is large enough to accommodate long and bulky alkyl groups. Substitution of C8 in **24** with a nitrogen atom to produce **21** resulted also in a good ADA inhibitor, although slightly weaker than **24**.

Apparently, C2-hexylthio substitution in **21** improved its affinity to the enzyme 15-fold as compared to 8-aza-adenosine,[54] implying that the hydrophobic interaction of the C2 substituent is a significant recognition determinant.

2-Substituted Nebularine Analogues. C2-substituted-nebularine analogues, **11**−**14**, proved efficient ADA inhibitors, with K_i values ranging from 3 to 76 μM (Table 2). This result is probably due to their predominant *anti* conformation and *N* sugar puckering preferred by ADA as well as preferred (69%) *gg* C4′−C5′ rotamer (e.g., **12** in Figure 5) in addition to favorable hydrophobic interactions as those observed for C2-substituted adenosine and 8-aza-adenosine analogues **20**−**25**.

2-Chloro-nebularine was found to be a moderate ADA inhibitor (K_i 37 μM), slightly more potent than the corresponding

2-chloro adenosine.[76] Because 2-Cl-nebularine proved a less potent inhibitor as compared to the parent compound, nebularine (K_i 37 vs 7−22 μM,[26−29] respectively), we prepared and evaluated nebularine analogues substituted with electron donating groups at C2, analogues **12**−**14**.

The most potent 2-substituted nebularine analogue was 2-SMe-nebularine, **12** (K_i 3 μM). 2-MeNH and 2-BnNH nebularine analogues, **13**−**14**, proved to be less potent (K_i 76 and 46 μM, respectively). Again, as found for 2-substituted adenosine analogues, i.e., 2-MeNH vs 2-nPrNH-adenosine analogues[76] (Table 2), the bulkiest substitution at the nebularine C2 provides the most potent analogue. Thus, 2-BnNH nebularine is more potent than 2-MeNH nebularine analogue. The higher activity of **12** vs **13** is probably due to a tighter binding to ADA with the larger sulfur atom of **12**, similar to 2-nPrS-adenosine, which was a better inhibitor than 2-nPrNH-adenosine.[76] Additional interactions between 2-MeS-nebularine and the enzyme are possible, such as H-bonding of the sulfur atom with an H-donor in the binding site.[85] The greater size of the sulfur atom and its nπ lone pair donor character may explain the increased activity of 2-MeS-nebularine over that of 2-MeNH-nebularine. Furthermore, the C2-thiomethyl group is expected to be surrounded by many hydrophobic amino acids (e.g., Phe61 and Leu58) based on X-ray crystal structure of ADA.[39,86] Such a hydrophobic pocket may explain the fact that increasing the length of the thioether side chain in 2-*RS*-adenosine nucleosides, **22**−**25**, enhances the affinity toward ADA. Thus, we hypothesize that both the substituent heteroatom and the alkyl group play important roles in the recognition of 2-substituted adenosine derivatives by ADA rather than changes in the charge distribution of the adenine ring. The above hypothesis cannot explain the activity of 2-Cl-adenosine. However, like 2-MeS-adenosine, this compound might be moved into a more favorable position and the large Cl atom may give rise to a tighter fit between nucleoside and the enzyme. Furthermore, the increased H-bonding donor character of the N^6-hydrogens of the 2-Cl- derivative, as seen by the MEP,[38] may explain enhanced interactions with the enzyme.

Another structural factor is γ angle. The *gg* rotamer was observed in the crystal structure of ADA with different inhibitors.[26,39,41,86] Indeed, **12**, **48**, and **21**, which are potent ADA inhibitors, have a preference for *gg* rotamer in solution (68.5% and 61.6%, respectively). However, **18**, which is a very weak inhibitor, prefers the *gt* conformer. Thus, a preference for the *gg* rotamer is probably a major recognition determinant.[84]

The only nebularine analogues tested as ADA inhibitors thus far are 8-aza- and 2-amino nebularine. Both of these analogues are good inhibitors of ADA (Table 2) (0.04 and 4.2 μM, respectively).[26,87] We tested both 8- and 2-substituted nebularine analogues and compared them to adenosine analogues. 8-Aza-nebularine is a much better inhibitor than its adenosine analogue (Table 2) (0.04 and 145 μM, respectively).[26,54] Similarly, we observed that 2-MeS-nebularine is a better inhibitor than 2-MeS-adenosine (3 and 33 μM, respectively). Thus, nebularine analogues are more tightly bound than adenosine counterparts. This may be due to hydration of the nebularine analogues at C6, thus forming transition state analogue similarly to 6-hydroxy-1,6-dihydropurine riboside (HDPR).[88,89] Adenosine analogues **20**−**25** are inhibitors of ADA and are not hydrolyzed, possibly due to the shift of C6-NH_2 away from the essential water molecule.

The most potent inhibitor we have identified is 2-MeS-nebularine (K_i 3 μM). This analogue adopts the conformation preferred by ADA, and its 2-MeS-substituent perfectly fits the hydrophobic pocket of ADA. 2-MeS-nebularine is active in the micromolar range and is expected to be less toxic than known inhibitors such as coformycin.

Conclusion

All 8-substituted purine riboside analogues proved to be nonsubstrates and noninhibitors of ADA. 2-Substituted purine riboside as well as 2-substituted adenosine and 8-aza-adenosine analogues proved efficient ADA inhibitors, with K_i values in the micromolar range. The most potent inhibitor of this series is 2-SMe-purine riboside with K_i of 3 μM. Correlation was found between the nucleoside conformation and inhibitory activity of ADA. Modest to potent inhibitors based on 2-substituted nebularine/adenosine/8-aza-adenosine adopt preferentialy *anti*, *S*(*N*), and *gg* conformation. On the other hand, 8-substituted-purine riboside analogues adopting *syn*, *S*, and *gt* conformation are not recognized by ADA, while 8-substituted-adenosine analogues adopting *syn*, *S*, and *gg* conformation are modest ADA inhibitors.

These findings might imply that conformational parameters such as *syn*/*anti* or *S*/*N* are not important recognition determinants, while a *gg* rotamer about C4′−C5′ bond is required for recognition by ADA. In addition to the nucleoside conformation, the complementarity of the C2 substituent with ADA's hydrophobic pocket is another significant recognition determinant. Specifically, the presence of a large heteroatom at C2, which gives rise to a tighter fit with the binding pocket, improves binding interactions with the enzyme.

Experimental Section

General. All air- and moisture-sensitive reactions were carried out in flame-dried, argon-flushed, two-necked flasks sealed with rubber septa, and the reagents were introduced with a syringe. TLC analysis was performed on precoated Merck silica gel plates (60F-254). Visualization was accomplished by UV light. All compounds were separated on a MPLC system (Biotage, Kungsgatan, Uppsala, Sweden) using a silica gel column (12 + M, 25 + M, or 40 + M column) using gradient schemes as indicated below for each compound. The purity of the nucleotides was evaluated on an analytical reverse-phase column system (Gemini 5μ, C-18, 110A, 150 mm × 4.60 mm, 5 μm, Phenomenex, Torrance, CA) in two solvent systems as described below. The purity of the nucleosides evaluated was generally ≥95%. NMR spectra were recorded on Bruker AC 200 instrument (200 and 50.3 MHz for ^{1}H, ^{13}C respectively) or DPX-300 instrument (300.1 and 75.5 30.4 MHz for ^{1}H and ^{13}C, respectively) or on a DMX-600 instrument (600.1, and 150.9 MHz for ^{1}H and ^{13}C, respectively). ^{1}H NMR and ^{13}C NMR were recorded in CD_3OD or $CDCl_3$ as indicated for each compound. The chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as an internal standard. High resolution mass spectra were recorded on an AutoSpec-E FISION VG mass spectrometer by chemical ionization. All commercial reagents were used without further purification unless otherwise noted. All reactants in moisture sensitive reactions were dried overnight under high in a vacuum oven. pH measurements were performed with an Orion microcombination pH electrode and a Hanna Instruments pH meter. Compounds **15** and **18**−**19** were prepared according to literature procedure.[60] Likewise, the synthesis of compounds **22**−**25** and **48** was based on literature.[75]

(2*R*,3*R*,4*S*,5*R*)-2-(2-Chloro-9*H*-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol (11). (2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(2-chloro-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate,

27 (328.8 mg, 0.8 mmol), was dissolved in chloroform (2 mL) and 7 N NH_3 solution in methanol was added (10 mL, 70 mmol, 88 equiv). The pressure vessel was sealed and kept overnight at 4 °C. TLC (4:1 $CHCl_3$:MeOH) indicated that no starting material left. The volatiles were evaporated, and the residue was separated on silica gel column using 9:1 $CHCl_3$:MeOH. The resulting oil was crystallized with chloroform. The product was obtained in an 89% yield (204 mg, 0.71 mmol). Spectroscopic data are consistent with reported values.[56]

(2*R*,3*S*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(2-(methylamino)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (13). (2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(2-chloro-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate, **27** (102.8 mg, 0.25 mmol), was dissolved in dry ethanol (6 mL) and solution of 33% NH_2Me in ethanol (2 mL, 50 mmol, 100 equiv) was added. The mixture was sealed in a pressure vessel and heated at 70 °C for 3 h. TLC (8:2 $CHCl_3$: MeOH) indicated that no starting material was left. The volatiles were evaporated, and the residue was separated on a MPLC system using a silica gel column (12 + M column) and the following gradient scheme: 2 column volumes (CV) of 75:15 (A) $CHCl_3$ (B) MeOH, 8 CV of a gradient from 75:15 to 60:40 of A:B and 2 CV of 60:40 A:B at a flow rate of 12 mL/min. The product was obtained in a 38% yield (27 mg, 0.095 mmol). Purity was determined using HPLC applying two solvent systems: (1) a linear gradient of H_2O/CH_3CN 20:80 to 2:98 in 7 min (flow rate 1 mL/min), t_R 5 min (98% purity); (2) linear gradient of H_2O/MeOH 30:70 to 2:98 in 5 min (flow rate 1 mL/min), t_R 4.3 min (96% purity). ^{1}H NMR (CD_3OD, 200 MHz) δ: 8.57 (s, 1H, H8), 8.26 (s, 1H, H6), 5.98 (d, J = 5.2 Hz, 1H, H1′), 4.8 (dd, J = 5.2, 3.8 Hz, 1H, H2′), 4.38 (dd, J = 5, 3.8 Hz, 1H, H3′), 4.1 (ddd, J = 5, 4, 3.4 Hz, 1H, H4′), 3.85 (dd, J = 13.5, 3.4 Hz, 1H, H5′), 3.75 (dd, J = 11.6, 4 Hz, 1H, H5″), 2.92 (s, 3H, CH_3) ppm. ^{13}C NMR δ: 152.8 (C-2), 151.6 (C-4), 149.3 (C-6), 144.1 (C-8), 127 (C-5), 89.2 (C-1′), 86.4 (C-4′), 72.85 (C-3′), 74.6 (C-2′), 62.1 (C-5′), 28.5 (CH_3) ppm. MS TOF ES$^+$: 282 (M^- H^+), 303 (MNa^+).

(2*R*,3*R*,4*S*,5*R*)-2-(8-Amino-9*H*-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol (16). (2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(8-bromo-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate, **28** (200 mg, 0.44 mmol), was dissolved in 7 N ammonia in methanol (8 mL, 56 mmol, 127 equiv), and the pressure vessel was sealed. The solution was stirred at RT overnight. The volatiles were evaporated, and the resulting mixture was separated on silica column using 10:1 $CHCl_3$:MeOH and 1% acetic acid. The product was obtained as a beige solid in a 37% yield (87.2 mg). Spectroscopic data are consistent with reported values.[60]

(2*R*,3*S*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(8-(methylamino)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (17). (2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(8-bromo-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate, **28** (260.2 mg, 0.57 mmol), was dissolved in ethanol (5 mL) followed by addition of methylamine solution in ethanol (33%) (3 mL, 2.85 mmol, 5 equiv). After stirring at 0 °C for 2.5 h, the volatiles were evaporated. The product was obtained as yellowish solid (38.1 mg, 0.14 mmol) in a 24% yield. Purity was determined using HPLC applying two solvent systems: (1) a linear gradient of H_2O/CH_3CN 20:80 to 2:98 in 5 min and then 2:98 for 5 min (flow rate 1 mL/min), t_R 5.5 min (99% purity); (2) linear gradient of H_2O/MeOH 30:70 to 2:98 in 5 min and then 2:98 for 5 min (flow rate 1 mL/min), t_R 7 min (97% purity). ^{1}H NMR (CD_3OD, 300 MHz) δ: 8.6 (s, 1H, H2), 8.5 (s, 1H, H6), 6.1 (d, J = 6 Hz, 1H, H1′), 4.6 (dd, J = 6, 5 Hz, 1H, H2′), 4.2 (dd, J = 5, 3 Hz, 1H, H3′), 4.05 (ddd, J = 4, 3.6, 3 Hz, 1H, H4′), 3.8 (dd, J = 12, 3.6, 1H, H5′), 3.6 (dd, J = 12, 4, 1H, H5″), 2.9 (s, 3H, CH_3) ppm. ^{13}C NMR δ: 156.2 (C-6), 155.3 (C-8), 151.3 (C-2), 149.6 (C-4), 116.7 (C-5), 89.0 (C-1′), 88.2 (C-4′), 73.7 (C-2′), 71.84 (C-3′), 64 (C-5′) ppm. MS TOF ES$^+$: 282 (MH^+).

(2*R*,3*R*,4*S*,5*R*)-2-(7-Amino-5-(benzylthio)-3*H*-[1,2,3]triazolo-[4,5-*d*]pyrimidin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (20). (2*R*,3*R*,4*R*,5*R*)-2-(7-Amino-5-(benzylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]-pyrimidin-3-yl)-5-(benzoyloxymethyl)tetrahydrofuran-3,4-diyl dibenzoate, **41** (330 mg, 0.47 mmol), was dissolved in 2 M NH_3 in MeOH (12 mL), and the pressure vessel was sealed. The solution was stirred at RT for 18 h. The volatiles were evaporated and flash chromatographed on silica gel column using 99:1 $CHCl_3$:MeOH. Product was obtained in 23% yield (41.2 mg). Purity was determined using HPLC by applying two solvent systems: (1) a linear gradient of H_2O/CH_3CN 20:80 to 0:100 in 5 min (flow rate 1 mL/min), t_R 3 min (99% purity); (2) linear gradient of H_2O/MeOH 30:70 to 2:98 in 5 min and then 2:98 for 5 min (flow rate 1 mL/min), t_R 6.5 min (97% purity). ^{1}H NMR (CD_3OD, 300 MHz) δ: 6.3 (d, J = 4.8 Hz, 1H, H1′), 4.71 (dd, J = 5, 4.8 Hz, 1H, H2′), 4.54 (dd, J = 9.4, 5 Hz 1H, H3′), 4.42 (ddd, J = 9.4, 4.4, 2.8 Hz, 1H, H4′), 3.9 (dd, J = 12, 2.8 Hz, 1H, H5′), 3.8 (dd, J = 12, 4.4 Hz, 1H, H5″), 3.3 (m, 2H, SCH_2) ppm. ^{13}C NMR δ: 171.0 (C-2), 156.1(C-6), 151.0 (C-4), 137.1, 128.7, 127.7 (Bz), 123.9 (C-5), 104.5 (C-1′), 87.4 (C-4′), 72.6 (C-2′), 70.5 (C-3′), 61.6 (C-5′), 34.7 (SCH_2) ppm. MS TOF ES$^+$: 391 (MH^+).

(2*R*,3*R*,4*S*,5*R*)-2-(7-Amino-5-(hexylthio)-3*H*-[1,2,3]triazolo-[4,5-*d*]pyrimidin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (21). (2*R*,3*R*,4*R*,5*R*)-2-(7-Amino-5-(hexylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]-pyrimidin-3-yl)-5-(benzoyloxymethyl)tetrahydrofuran-3,4-diyl dibenzoate, **42** (109.9 mg, 0.158 mmol), was dissolved in 2 M NH_3 in MeOH (10 mL), and the pressure vessel was sealed. The solution was stirred at RT for 18 h. The volatiles were evaporated and the residue flash chromatographed on a silica gel column using 99:1 to 17:1 $CHCl_3$:MeOH. Product was obtained in a 71% yield (43 mg). Purity was determined using HPLC applying two solvent systems: (1) a linear gradient of H_2O/CH_3CN 20:80 to 2:98 in 5 min and then 2:98 for 5 min (flow rate 1 mL/min), t_R 4 min (95% purity); (2) linear gradient of H_2O/MeOH 30:70 to 2:98 in 5 min and then 2:98 for 5 min (flow rate 1 mL/min), t_R 6 min (95% purity). ^{1}H NMR (CD_3OD, 300 MHz) δ: 6.26 (d, J = 4.5 Hz, 1H, H1′), 4.93 (dd, J = 4.8, 4.5 Hz, 1H, H2′), 4.496 (dd, J = 4.8, 4.8 Hz, 1H, H3′), 3.85 (ddd, J = 4.8, 4.8, 3.6 Hz 1H, H4′), 3.82 (dd, J = 12.3, 3.6 Hz, 1H, H5′), 3.69 (dd, J = 12.3, 4.8 Hz, 1H, H5″), 3.145 (m, 2H, SCH_2), 1.696 (m, 2H, SCH_2CH_2), 1.48−1.278 (m, 9H), 0.9 (m, 3H, CH_3) ppm. ^{13}C NMR δ: 172.73 (C-2), 156.8 (C-6), 151.14 (C-4), 124.27 (C-5), 91.94 (C-1′), 89.31 (C-4′), 79.44 (C-2′), 72.56 (C-3′), 63.74 (C-5′), 32.58 (SCH_2), 32.02 (SCH_2CH_2), 30.72 ($SCH_2CH_2CH_2$), 30.50 ($SCH_2CH_2CH_2CH_2$), 23.63 $SCH_2CH_2CH_2CH_2CH_2$), 14.37 (CH_3) ppm. MS TOF ES$^+$: 384 (MH^+).

7-Amino-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine-5-disulfide (33). To a chilled solution, at 0 °C, of 4,5,6-triaminopyrimidine-2-thiol (103 mg, 0.66 mmol), **31**, in HCl:H_2O (1:6) was added sodium nitrite (73 mg, 1.06 mmol) in water (2 mL) during 15 min. The resulting greenish suspension was stirred at 0 °C for 3 h. The resulting suspension was neutralized using NH_4OH and the solid was isolated by suction filtration and washed with cold water. According TOF MS ES$^+$: 335 (MH^+).

2-(Benzylthio)pyrimidine-4,6-diamine (35). A suspension of 4,6-diaminopyrimidine-2-thiol, **34**[76] (4 g, 26.45 mmol in 120 mL MeOH), was dissolved in 0.25 M NaOH (111 mL) and stirred at RT for 1 h. The solvent was evaporated under high vacuum. The dry sodium thiolate salt was dissolved in dry DMF (80 mL), and benzyl bromide (3.35 mL, 28 mmol, 1.1 equiv) was added. The clear solution was stirred at room temperature overnight. TLC (9:1 $CHCl_3$:MeOH) indicated that no starting material was left. The solvent was evaporated under high vacuum. The product was obtained as yellow oil in a 89% yield (5.46 g). ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.38−7.24 (m, 5H, Bn), 4.3 (s, 2H, CH_2) ppm. ^{13}C NMR δ: 170.12 (C-2), 164.92 (C-4, C-6), 139.84 (SCH_2), 130.04, 129.5, 129.35 (Bz) ppm. MS TOF ES$^+$: 233 (MH^+). HRMS calcd for 232.0783, found 232.0796.

2-(Benzylthio)-5-nitrosopyrimidine-4,6-diamine (36). To a chilled solution, at 0 °C, of 2-(benzylthio)pyrimidine-4,6-diamine, **35** (6.66 g, 26.45 mmol), in acetic acid (77 mL) and water (25 mL) was added sodium nitrite (3.285 g, 47.6 mmol, 1.8 equiv) in water (10 mL) during 15 min. The resulting pink suspension was stirred at 0 °C for 15 min, and the product was isolated by suction filtration and washed with cold water. The filter cake was dried to give product as a gray−blue solid, which was recrystallized from hot methanol to give the product as blue solid in 86% yield (5.9 g). ^{1}H NMR

(CD_3OD, 300 MHz) δ: 8.01−7.23 (m, 5H, Bn), 4.45 (s, 2H, CH_2) ppm. ^{13}C NMR δ: 177.1 (C-2), 166.0 (C-5), 148.6 (C-4), 139.7 (C-6), 137.1, 128.7, 127.7 (Bz), 34.6 (SCH_2) ppm. MS TOF ES$^+$: 262 (M^-H^+). HRMS, calcd for $C_{14}H_7N_5OS$ 261.0684 (MH^+), found 261.0652.

2-(Benzylthio)pyrimidine-4,5,6-triamine (37). 2-Benzylthio-5-nitrosopyrimidine-4,6-diamine, **36** (2.079 g, 7.96 mmol), dissolved in EtOH (50 mL) was hydrogenated at atmospheric pressure, for 6 h, at RT, over PtO_2 catalyst. The reaction mixture was filtered through a bed of Celite. The filtrate was concentrated and dried to give product as green oil, in a quantitative yield. ^1H NMR (CD_3OD, 300 MHz) δ: 7.49−7.1 (m, 5H, Bn), 4.31 (s, 2H, CH_2) ppm. ^{13}C NMR δ: 162.0 (C-2), 155.4 (C-4, C-6), 137.1−127.6 (Bz), 108.9 (C-5) ppm. HRMS: calcd for $C_{11}H_{13}N_5S$ 247.0892 (MH^+), found 247.0862.

5-(Benzylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-amine (39). 2-Benzylthiopyrimidine-4,5,6-triamine, **37** (14.7 mmol), was dissolved in 30% HCl and a solution of sodium nitrite (1.65 g, 23.9 mmol, 1.6 equiv) in water (20 mL) was added dropwise. A green solid precipitated during the addition of sodium nitrite. The suspension was stirred for 3 h at 0 °C. The reaction mixture was extracted with ethyl acetate. Organic phase was dried over $MgSO_4$, filtered, and the volatiles were evaporated. The product was obtained as a green solid in a 55% yield (2.09 g). ^1H NMR (CD_3OD, 300 MHz) δ: 7.8−7.3 (m, 5H, Bn), 4.43 (t, 2H, SCH_2) ppm. ^{13}C NMR δ: 171.0 (C-2), 159.9 (C-4), 156.1 (C-6), 137.1, 128.7, 127.7 (Bn), 122.9 (C-5), 34.7 (SCH_2) ppm. MS TOF ES$^+$: 259 (M^-H^+). HRMS: calcd for $C_{14}H_6N_6S$ (M^+) 258.0688, found 258.0695.

5-(Hexylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-amine (40). 2-(Hexylthio)pyrimidine-4,5,6-triamine, **38**[76] (9.73 mmol), was dissolved in 30% HCl and a solution of sodium nitrite (1.08 g, 15.7 mmol, 1.6 equiv) in water (5 mL) was added dropwise. A green solid was precipitated during the addition of sodium nitrite. The suspension was stirred for 3 h at 0 °C. The reaction mixture was extracted with ethyl acetate (5 times), organic phase was dried over $MgSO_4$, and the volatiles were evaporated. The product was obtained as a green solid in a 73% yield (1.8 g). ^1H NMR (CD_3OD, 600 MHz) δ: 2.93 (m, 2H, SCH_2), 1.456 (m, 2H, SCH_2CH_2), 1.9 (m, 2H, $SCH_2CH_2CH_2$), 1.04 (m, 4H, $SCH_2CH_2CH_2CH_2CH_2$), 0.65 (t, J = 7 Hz, 3H, CH_3) ppm. ^{13}C NMR δ: 165.39 (C-2), 160.63 (C-6), 155.66 (C-4), 122.89 (C-5), 31.76 (SCH_2), 31.3 (SCH_2CH_2), 29.93 ($SCH_2CH_2CH_2$), 29.58 ($SCH_2CH_2CH_2CH_2$), 22.85 ($SCH_2CH_2CH_2CH_2CH_2$), 14.24 (CH_3) ppm. MS TOF ES$^+$: 253 (MH^+). HRMS: calcd for $C_{10}H_{13}N_6S$ (M^+) 253.1202, found 253.1210.

(2*R*,3*R*,4*R*,5*R*)-2-(7-Amino-5-(benzylthio)-3*H*-[1,2,3]triazolo-[4,5-*d*]pyrimidin-3-yl)-5-(benzoyloxymethyl)tetrahydrofuran-3,4-diyl Dibenzoate (41). A solution of 5-(benzylthio)-3*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-7-amine, **39** (530 mg, 2.05 mmol), and $(NH_4)_2SO_4$ (catalytic amount) in freshly distilled HMDS (16 mL) was stirred under reflux for 2 h under argon atmosphere. The solution have been cooled down to room temperature and evaporated under high vacuum. A solution of 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (1.04 g, 2.05 mmol, 0.98 equiv) in freshly distilled 1,2-DCE (10 mL) was added to the oily residue of the silylated base, followed by a solution of trimethylsilyl triflate (3 mL, 16.6 mmol, 2.3 equiv) in 1,2-DCE (10 mL). The mixture was heated under reflux for 2 h. TLC (9:1 $CHCl_3$:MeOH) indicated that no starting material was left. The reaction mixture was diluted with methylene chloride and washed with saturated $NaHCO_3$ solution and brine. The organic phase was dried with $MgSO_4$, filtered, concentrated, and flash chromatographed on silica gel column using 99.7:0.3 $CHCl_3$:MeOH. Product was obtained in a 23% yield (333.4 mg). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.15−7.97 (m, 3H, Bz), 7.6 (m, 5H, Bn), 7.43 (m, 6H, Bz) 7.39−7.36 (m, 6H, Bz), 6.81 (d, J = 2.7 Hz, 1H, H1′), 6.6 (dd, J = 5.4, 2.7 Hz, 1H, H2′), 6.43 (dd, J = 6.6, 5.4 Hz,1H, H3′), 4.97 (ddd, J = 6.6, 5.4, 4 Hz, 1H, H4′), 4.81 (dd, J = 13, 4 Hz, 1H, H5′), 4.64 (dd, J = 13, 5.4 Hz, 1H, H5″), 4.52 (s, 2H, SCH_2) ppm. ^{13}C NMR δ: 171.66 (C-2), 166.16, 165.15, 165.03 (CO), 154.81 (C-6), 150.10 (C-4), 137.00 (C-5), 133.78, 133.58, 129.87, 129.77, 129.36, 129.04, 128.71, 128.55, 128.46, 128.37, 127.18, 122.97 (Bz), 87.77 (C-1′), 80.35 (C-4′), 74.33 (C-2′), 771.81 (C-4′), 63.62 (C-5′), 35.72 (SCH_2) ppm. MS TOF ES$^+$: 703 (MH^+). HRMS calcd for $C_{37}H_{30}N_6O_7S$ 702.1885, found 702.1897.

(2*R*,3*R*,4*R*,5*R*)-2-(7-Amino-5-(hexylthio)-3*H*-[1,2,3]triazolo-[4,5-*d*]pyrimidin-3-yl)-5-(benzoyloxymethyl)tetrahydrofuran-3,4-diyl Dibenzoate (42). A solution of 5-(hexylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-amine, **40** (813.6 mg, 3.23 mmol), and $(NH_4)_2SO_4$ (catalytic amount) in freshly distilled HMDS (38 mL) was stirred under reflux for 1 h under nitrogen atmosphere. The solution was cooled down to room temperature and evaporated under high vacuum. A solution of 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (1.6 g, 3.17 mmol, 0.98 equiv) in freshly distilled 1,2-DCE (10 mL) was added to the oily residue of the silylated base, followed by a solution of trimethylsilyl triflate (1.37 mL, 7.6 mmol, 2.3 equiv) in 1,2-DCE (10 mL). The mixture was heated under reflux for 2 h, at which time TLC indicated a complete consumption of the sugar. The reaction mixture was diluted with methylene chloride and washed with saturated $NaHCO_3$ solution and brine. The organic phase was dried with $MgSO_4$, filtered, concentrated, and flash chromatographed on silica gel column using 99:1 $CHCl_3$:MeOH. Product was obtained in a 36% yield (798.2 mg). ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.99−7.97 (m, 3H, Bz), 7.57−7.53 (m, 6H, Bz) 7.39−7.36 (m, 6H, Bz), 6.72 (d, J = 3 Hz, 1H, H1′), 6.53 (dd, 1H, J = 5, 3 Hz, H2′), 6.38 (dd, J = 7, 5 Hz, 1H, H3′), 4.9 (ddd, J = 7, 5, 4.5 Hz, 1H, H4′), 4.79 (dd, J = 12, 5 Hz,1H, H5′), 4.64 (dd, J = 12, 4.5 Hz, 1H, H5″), 3.22 (m, 2H, SCH_2), 1.72 (m, 2H, SCH_2CH_2), 1.41 (m, 2H, $SCH_2CH_2CH_2$), 1.3 (m, 2H, $SCH_2CH_2CH_2CH_2$), 1.2 (m, 2H, CH_2CH_3), 0.82 (t, J = 7 Hz, 3H, CH_3) ppm. ^{13}C NMR δ: 171.99 (C-2), 166.13, 165.10, 164.97 (CO), 154.41 (C-6), 151.5 (C-4), 133.79, 133.61, 133.24, 129.93, 129.87, 129.82, 128.60, 128.51, 128.42 (Bz), 88.11 (C-1′), 80.44 (C-4′), 74.3 (C-2′), 71.71 (C-4′), 63.60 (C-5′), 31.42 (SCH_2), 31.34 ($SCH_2CH_2CH_2CH_2$), 28.79 (SCH_2CH_2), 28.52 ($SCH_2CH_2CH_2$), 22.64 (CH_2CH_3), 13.34 (CH_3) ppm. MS (FAB$^+$) 697 (MH^+). HRMS calcd for $C_{36}H_{36}N_6O_7S$ 696.2385 (M^+), found 696.2366.

(2*R*,3*R*,4*R*,5*R*)-2-(Benzoyloxymethyl)-5-(7-chloro-5-(hexylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)tetrahydrofuran-3,4-diyl Dibenzoate (44). BTEA-NO_2[76] (2.023 g, 8.5 mmol) was dissolved in dry CH_2Cl_2 (17 mL) and added dropwise (1 drop/2 s) to the cold (0 °C), stirred solution of AcCl (765 μL, 0.85 g, 10.8 mmol) in CH_2Cl_2 (8.5 mL). A cold solution of nucleoside **42** (1.2 g, 1.7 mmol) in dried CH_2Cl_2 (17 mL) was then added dropwise to the cooled, stirred AcCl/BTEA-NO_2/CH_2Cl_2 solution. After 4 h, TLC (9:1 CH_3Cl:MeOH) indicated completion of the reaction. Then the cold reaction mixture was added dropwise to mixture of CH_2Cl_2 (200 mL) and saturated $NaHCO_3$ (200 mL). The aqueous phase was extracted (CH_2Cl_2, 2 × 100 mL), and the combined organic phase was dried ($MgSO_4$) and filtered. The volatiles were evaporated, and the residue was separated on a MPLC system using a silica gel column (40 + M column) and the following gradient scheme: 2 column volumes (CV) of 98:2 (A) CH_2Cl_2 (B) MeOH, 8 CV of a gradient from 98:2 to 80:20 of A:B and 2 CV of 80:20 A:B at a flow rate of 40 mL/min. The product was obtained in a 59% yield (720 mg, 1 mmol). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.03−8.00 (m, 3H, Bz), 7.60−7.58 (m, 6H, Bz) 7.46−7.3 (m, 6H, Bz), 6.8 (d, J = 2.6 Hz, 1H, H1′), 6.60 (dd, J = 5.5, 2.6 Hz 1H, H2′), 6.39 (dd, J = 6.8, 5.5 Hz, 1H, H3′), 5.10 (ddd, J = 6.8, 5, 4 Hz, 1H, H4′), 4.82 (dd, J = 12, 4 Hz, 1H, H5′), 4.71 (dd, J = 12, 5 Hz, 1H, H5″), 3.31 (s, 2H, SCH_2), 1.82−1.77 (m, 2H, SCH_2CH_2), 1.35−1.30 (m, 6H), 0.9 (m, 3H, CH_3) ppm. ^{13}C NMR (200 MHz) δ: 173.17 (C-2), 166.04, 165.10, 165.00 (CO), 153.40 (C-6), 150.70 (C-4), 133.87, 133.68, 133.26, 129.85, 129.73, 129.34, 128.59,

128.53, 128.43 (Bz), 88.48 (C-1′), 80.78 (C-4′), 74.25 (C-2′), 71.59 (C-4′), 63.30 (C-5′), 31.99 (SCH_2), 31.29 ($SCH_2CH_2CH_2CH_2$), 29.69 (SCH_2CH_2), 28.45 ($SCH_2CH_2CH_2$), 24.48 (CH_2CH_3), 14.07 (CH_3) ppm. TOF MS ES$^+$ 715, 717 (MH^+). HRMS: calcd for $C_{36}H_{35}N_5O_7SCl$ 716.1935 (M^+), found 716.1946.

(2*R*,3*R*,4*R*,5*R*)-2-(Benzoyloxymethyl)-5-(5-(hexylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)tetrahydrofuran-3,4-diyl Dibenzoate[76] (46). (2*R*,3*R*,4*R*,5*R*)-2-(Benzoyloxymethyl)-5-(7-chloro-5-(hexylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)-tetrahydrofuran-3,4-diyl dibenzoate, **44**, (940 mg, 1.32 mmol) was dissolved in dried ethanol (30 mL), and NaOAc (100 mg) was added. This solution was hydrogenated at 1 atm for 24 h. TLC (8:2 Hex:EtOAc) indicated that no starting material was left. The solvent was evaporated, and the residue was separated on a MPLC system using a silica gel column (12 + M column) and the following gradient scheme: 2 column volumes (CV) of 90:10 (A) Hex (B) EtOAc, 6 CV of a gradient from 90:10 to 100 of B at a flow rate of 12 mL/min. The product was obtained as a beige solid in 20% yield (180 mg). ^{1}H NMR (CD_3OD, 200 MHz) δ: ($CDCl_3$, 300 MHz) δ: 9.27 (s, 1H, H6), 8.01−7.96 (m, 3H, Bz), 7.96−7.95 (m, 6H, Bz) 7.56−7.50 (m, 6H, Bz), 6.85 (d, J = 2.7 Hz, 1H, H1′), 6.56 (dd, J = 6.3, 2.7 Hz, 1H, H2′), 6.39 (dd, J = 6.6, 6.3 Hz, 1H, H3′), 4.93 (ddd, J = 6.6, 4.2, 3 Hz, 1H, H4′), 4.83 (dd, J = 12.3, 4.2 Hz, 1H, H5′), 4.68 (dd, J = 12.3, 3 Hz, 1H, H5″), 3.30 (s, 2H, SCH_2), 1.82−1.77 (m, 2H, SCH_2CH_2), 1.32−1.26 (m, 6H), 0.9 (m, 3H, CH_3) ppm. ^{13}C NMR (200 MHz) δ: 171.92 (C-2), 166.04, 165.10, 165.00 (CO), 159.72 (C-6), 152.30 (C-4), 133.87, 133.68, 133.26, 129.85, 129.73, 128.9, 128.60, 128.53, 128.43 (Bz), 88.48 (C-1′), 80.78 (C-4′), 74.25 (C-2′), 71.59 (C-4′), 63.30 (C-5′), 36.72 (SCH_2), 32.40 ($SCH_2CH_2CH_2CH_2$), 31.60 (SCH_2CH_2), 30.45 ($SCH_2CH_2CH_2$), 22.70 (CH_2CH_3), 14.10 (CH_3) ppm. TOF MS ES$^+$ 682 (MH^+). HRMS calcd for $C_{36}H_{41}N_5O_7S$ 681. 2364 (M^+), found 681.2378.

Adenosine Deaminase Inhibition Assays. A 3.2 M suspension of adenosine deaminase (ADA) from calf intestine (Roche Diagnostics Corporation) in $(NH_4)_2SO_4$ solution was exchanged for 50 mM K_2HPO_4 buffer, pH 7, by repeatedly concentrating the sample to less than 0.5 mL and rediluting to 2 mL with fresh buffer in a distilled-water-rinsed Amicon Centricon-10 centrifuge concentrator. Six exchange cycles were repeated with the phosphate buffer.

ADA reactivity was assayed as follows:[76] 0.1 mL of 1.4 mM adenosine solution in 0.05 M phosphate buffer, pH 7.4, was added to ice-cold 2.88 mL solution of ca. 0.8 units/mL of ADA in 0.05 M phosphate buffer, pH 7.4. The decrease in the UV absorbance of adenosine (at 265 nm) due to its deamination was measured as a function of time and the enzyme activity was found to be 146 units/mg.

The target compounds were screened against calf spleen ADA in vitro in a 50 mM phosphate buffer (pH 7.4) at 25 °C. The rate of hydrolysis of adenosine to inosine was monitored spectrophotometrically by following the absorbance decrease over 2 min at 265 nm using a 1 cm path length cuvette. The enzyme and substrate stock solution were prepared in a 50 mM phosphate buffer. While holding the enzyme and inhibitor concentration constant, the substrate concentration was varied to obtain the kinetic data. The decrease in substrate UV absorption in each assay was measured using four different concentrations of adenosine ranging from 10 to 100 μM. This was repeated with a different inhibitor concentration, ranging from 20 to 100 μM. In each assay, the inhibitors were used without preincubation with enzyme. The substrate and inhibitors were added to the phosphate buffer, and the reaction was started by addition of enzyme (0.08 units/mL). Assays were performed in duplicate. Lineweaver−Burk ($1/V$ vs $1/[S]$) plots were constructed for each assay and K_i was obtained as the intercept with X axis in the plot of $[I]$ (inhibitor concentration) vs slope of double reciprocal plot.

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References

(1) Cristalli, G.; Costanzi, S.; Lambertucci, C.; Lupidi, G.; Vittori, S.; Volpini, R.; Camaioni, E. Adenosine deaminase: functional implications and different classes of inhibitors. *Med. Res. Rev.* **2001**, *21*, 105–128.
(2) Blackburn, M. R.; Kellems, R. E.; Frederick, W. A. Adenosine deaminase deficiency: metabolic basis of immune deficiency and pulmonary inflammation. In *Advances in Immunology*; Academic Press: New York, 2005; pp 1−41.
(3) Hershfield, M. S. New insights into adenosine-receptor-mediated immunosuppression and the role of adenosine in causing the immunodeficiency associated with adenosine deaminase deficiency. *Eur. J. Immunol.* **2005**, *35*, 25–30.
(4) Poursharifi, P.; Saghiri, R.; Ebrahimi-Rad, M.; Nazem, H.; Pourpak, Z.; Moin, M.; Shams, S. Adenosine deaminase in patients with primary immunodeficiency syndromes: The analysis of serum ADA1 and ADA2 activities. *Clin. Biochem.* **2009**, *42*, 1438–1443.
(5) Reilly, K. M.; Kisor, D. F. Profile of nelarabine: use in the treatment of T-cell acute lymphoblastic leukemia. *OncoTargets Ther.* **2009**, *2*, 219–228.
(6) Czuczman, M. S.; Porcu, P.; Johnson, J.; Niedzwiecki, D.; Kelly, M.; Hsi, E. D.; Cook, J. R.; Canellos, G.; Cheson, B. D. Results of a phase II study of 506U78 in cutaneous T-cell lymphoma and peripheral T-cell lymphoma: CALGB 59901. *Leuk. Lymphoma* **2007**, *48*, 97–103.
(7) Akkari, R.; Burbiel, J. C.; Hockemeyer, J.; Müller, C. E. Recent progress in the development of adenosine receptor ligands as antiinflammatory drugs. *Curr. Top. Med. Chem.* **2006**, *6*, 1375–1399.
(8) Blay, J.; Hoskin, D. W. Impaired lymphocyte activation in the presence of adenosine: mechanisms and physiologic relevance. *Adenosine Recept.* **2007**, 69–88.
(9) Haskó, G.; Cronstein, B. N. Adenosine: an endogenous regulator of innate immunity. *Trends Immunol.* **2004**, *25*, 33–39.
(10) Ho, A. D.; Hensel, M. Pentostatin for the treatment of indolent lymphoproliferative disorders. *Semin. Hematol.* **2006**, *43*, S2–S10.
(11) Dearden, C. E. Role of single-agent purine analogues in therapy of peripheral T-cell lymphomas. *Semin. Hematol.* **2006**, *43*, S22–S26.
(12) Cass, C. E.; Au-Yeung, T. H. Enhancement of 9-β-D-arabinofuranosyladenine cytotoxicity to mouse leukemia L1210 in vitro by 2′-deoxycoformycin. *Cancer Res.* **1976**, *36*, 1486–1491.
(13) Johns, D. G.; Adamson, R. H. Enhancement of the biological activity of cordycepin (3′-deoxyadenosine) by the adenosine deaminase inhibitor 2′-deoxycoformycin. *Biochem. Pharmacol.* **1976**, *25*, 1441–1444.
(14) Adamson, R. H.; Zaharevitz, D. W.; Johns, D. G. Enhancement of the biological activity of adenosine analogs by the adenosine deaminase inhibitor 2′-deoxycoformycin. *Pharmacology* **1977**, *15*, 84–89.
(15) Glazer, R. I.; Lott, T. J.; Peale, A. L. Potentiation by 2′-Deoxycoformycin of the Inhibitory Effect by 3′-Deoxyadenosine (Cordycepin) on Nuclear RNA Synthesis in L1210 Cells in Vitro. *Cancer Res.* **1978**, *38*, 2233–2238.
(16) Agarwal, R. P.; Parks, R. E. Adenosine deaminase from human erythrocytes. In *Methods in Enzymology*; Hoffe, P. A., Jones, M. E., Eds.; Academic Press: Oxford, 1978; pp 502−507.
(17) Franco, R.; Pacheco, R.; Gatell, J. M.; Gallart, T.; Lluis, C. Enzymatic and extraenzymatic role of adenosine deaminase 1 in T-cell-dendritic cell contacts and in alteration of the immune function. *Crit. Rev. Immunol.* **2007**, *27*, 495–509.
(18) Genini, D.; Adachi, S.; Chao, Q.; Rose, D. W.; Carrera, C. J.; Cottam, H. B.; Carson, D. A.; Leoni, L. M. Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. *Blood* **2000**, *96*, 3537–3543.
(19) Asakura, M.; Asanuma, H.; Kim, J.; Liao, Y.; Nakamaru, K.; Fujita, M.; Komamura, K.; Isomura, T.; Furukawa, H.; Tomoike, H.; Kitakaze, M. Impact of adenosine receptor signaling and metabolism on pathophysiology in patients with chronic heart failure. *Hypertens. Res.* **2007**, *30*, 781–787.
(20) Hisatome, I. Adenosine and cardioprotection in chronic heart failure: genes and protein expression. *Hypertens. Res.* **2007**, *30*, 757–758.
(21) Cha, S.; Agarwal, R. P.; Parks, R. E. Tight-binding inhibitors-II: non-steady state nature of inhibition of milk xanthine oxidase by allopurinol and alloxanthine and of human erythrocytic adenosine deaminase by coformycin. *Biochem. Pharmacol.* **1975**, *24*, 2187–2197.
(22) Agarwal, R. P.; Spector, T.; Parks, R. E., Jr. Tight-binding inhibitors. IV. Inhibition of adenosine deaminases by various inhibitors. *Biochem. Pharmacol.* **1977**, *26*, 359–367.

(23) Da Settimo, F.; Primofiore, G.; La Motta, C.; Taliani, S.; Simorini, F.; Marini, A. M.; Mugnaini, L.; Lavecchia, A.; Novellino, E.; Tuscano, D.; Martini, C. Novel, Highly Potent Adenosine Deaminase Inhibitors Containing the Pyrazolo[3,4-*d*]pyrimidine Ring System. Synthesis, Structure−Activity Relationships, and Molecular Modeling Studies. *J. Med. Chem.* **2005**, *48*, 5162–5174.
(24) Schaeffer, H. J.; Schwender, C. F. Enzyme inhibitors. 26. Bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl)adenines. *J. Med. Chem.* **1974**, *17*, 6–8.
(25) Cristalli, G.; Eleuteri, A.; Franchetti, P.; Grifantini, M.; Vittori, S.; Lupidi, G. Adenosine deaminase inhibitors: synthesis and structure activity relationships of imidazole analogs of erythro-9-(2-hydroxy-3-nonyl)adenine. *J. Med. Chem.* **1991**, *34*, 1187–1192.
(26) Shewach, D. S.; Krawczyk, S. H.; Acevedo, O. L.; Townsend, L. B. Inhibition of adenosine deaminase by azapurine ribonucleosides. *Biochem. Pharmacol.* **1992**, *44*, 1697–1700.
(27) Frederiksen, S. Specificity of adenosine deaminase toward adenosine and 2′-deoxyadenosine analogs. *Arch. Biochem. Biophys.* **1966**, *113*, 383–388.
(28) Ronca, G.; Zucchelli, G. Competitive inhibition of adenosine deaminase by purine and pyrimidine bases. *Biochim. Biophys. Acta, Enzymol.* **1968**, *159*, 203–205.
(29) Lupidi, G.; Riva, F.; Cristalli, G.; Grifantini, M. Inhibition of adenosine deaminase by deaza derivatives of adenosine and purine riboside. *Ital. J. Biochem.* **1982**, *31*, 396–403.
(30) La Motta, C.; Sartini, S.; Mugnaini, L.; Salerno, S.; Simorini, F.; Taliani, S.; Marini, A. M.; Da Settimo, F.; Lavecchia, A.; Novellino, E.; Antonioli, L.; Fornai, M.; Blandizzi, C.; Del Tacca, M. Exploiting the pyrazolo[3,4-*d*]pyrimidin-4-one ring system as a useful template to obtain potent adenosine deaminase inhibitors. *J. Med. Chem.* **2009**, *52*, 1681–1692.
(31) Major, P. P.; Agarwal, R. P.; Kufe, D. W. Deoxycoformycin: neurological toxicity. *Cancer Chemother. Pharmacol.* **1981**, *5*, 193–196.
(32) Major, P. P.; Agarwal, R. P.; Kufe, D. W. Clinical pharmacology of deoxycoformycin. *Blood* **1981**, *58*, 91–96.
(33) McConnell, W. R.; El Dareer, S. M.; Hill, D. L. Metabolism and disposition of erythro-9-(2-hydroxy-3-nonyl)[^{14}C]adenine in the rhesus monkey. *Drug Metab. Dispos.* **1980**, *8*, 5–7.
(34) Lambe, C. U.; Nelson, D. J. Pharmacokinetics of inhibition of adenosine deaminase by erythro-9-(2-hydroxy-3-nonyl)adenine in CBA mice. *Biochem. Pharmacol.* **1982**, *31*, 535–539.
(35) Reayi, A.; Hosmane, R. S. Inhibitors of adenosine deaminase: continued studies of structure−activity relationships in analogues of coformycin. *Nucleosides. Nucleotides Nucleic Acids* **2004**, *23*, 263–271.
(36) Terasaka, T.; Kinoshita, T.; Kuno, M.; Seki, N.; Tanaka, K.; Nakanishi, I. Structure-based design, synthesis, and structure−activity relationship studies of novel non-nucleoside adenosine deaminase inhibitors. *J. Med. Chem.* **2004**, *47*, 3730–3743.
(37) Major, D. T.; Laxer, A.; Fischer, B. Protonation studies of modified adenine and adenine nucleotides by theoretical calculations and ^{15}N NMR. *J. Org. Chem.* **2002**, *67*, 790–802.
(38) Major, D. T.; Halbfinger, E.; Fischer, B. Molecular recognition of modified adenine nucleotides by the P2Y1-receptor. 2. A computational approach. *J. Med. Chem.* **1999**, *42*, 5338–5347.
(39) Wang, Z.; Quiocho, F. A. Complexes of adenosine deaminase with two potent inhibitors: X-ray structures in four independent molecules at pH of maximum activity. *Biochemistry* **1998**, *37*, 8314–8324.
(40) Sharff, A. J.; Wilson, D. K.; Chang, Z.; Quiocho, F. A. Refined 2.5 Å structure of murine adenosine deaminase at pH 6.0. *J. Mol. Biol.* **1992**, *226*, 917–921.
(41) Wilson, D. K.; Rudolph, F. B.; Quiocho, F. A. Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. *Science* **1991**, *252*, 1278–1284.
(42) Orozco, M.; Canela, E. I.; Franco, R. A quantum chemical study of the enzymatic deamination of benzoadenine derivatives. *Eur. J. Biochem.* **1990**, *188*, 155–163.
(43) Marrone, T. J.; Straatsma, T. P.; Briggs, J. M.; Wilson, D. K.; Quiocho, F. A.; McCammon, J. A. Theoretical study of inhibition of adenosine deaminase by (8*R*)-coformycin and (8*R*)-deoxycoformycin. *J. Med. Chem.* **1996**, *39*, 277–284.
(44) Hosmane, R. S.; Hong, M. How important is the N-3 sugar moiety in the tight-binding interaction of coformycin with adenosine deaminase? *Biochem. Biophys. Res. Commun.* **1997**, *236*, 88–93.
(45) Gleeson, M. P.; Burton, N. A.; Hillier, I. H. The mechanism of adenosine deaminase catalysis studied by QM/MM calculations: The role of histidine 238 and the activity of the alanine 238 mutant. *Phys. Chem. Chem. Phys.* **2003**, *5*, 4272–4278.
(46) Il'icheva, I. A.; Zarubin, Y. P.; Kostin, P. A.; Mirgorodskii, D. V.; Purygin, P. P.; Florent'ev, V. L. Theoretical study of the structure of adenosine deaminase complexes with adenosine analogues: I. aza-, deaza-, and isomeric azadeazaanalogues of adenosine. *Bioorg. Khim* **2005**, *31*, 439–452.
(47) Hayatshahi, S. H. S.; Abdolmaleki, P.; Ghiasi, M.; Safarian, S. QSARs and activity predicting models for competitive inhibitors of adenosine deaminase. *FEBS Lett.* **2007**, *581*, 506–514.
(48) Deng, H.; Cahill, S.; Kurz, L.; Callender, R. The assignment of downfield proton resonances in an enzyme inhibitor complex using time-dependent saturation transferred NOEs. *J. Am. Chem. Soc.* **2004**, *126*, 1952–1953.
(49) Laxer, A.; Gottlieb, H. E.; Fischer, B. Molecular recognition of adenosine deaminase: ^{15}N NMR studies. *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 161–180.
(50) Sideraki, V.; Mohamedali, K. A.; Wilson, D. K.; Chang, Z.; Kellems, R. E.; Quiocho, F. A.; Rudolph, F. B. Probing the functional role of two conserved active site aspartates in mouse adenosine deaminase. *Biochemistry* **1996**, *35*, 7862–7872.
(51) Mohamedali, K. A.; Kurz, L. C.; Rudolph, F. B. Site-directed mutagenesis of active site glutamate-217 in mouse adenosine deaminase. *Biochemistry* **1996**, *35*, 1672–1680.
(52) Kinoshita, T.; Tada, T.; Nakanishi, I. Conformational change of adenosine deaminase during ligand exchange in a crystal. *Biochem. Biophys. Res. Commun.* **2008**, *373*, 53–57.
(53) Biagi, G.; Giorgi, I.; Livi, O.; Pacchini, F.; Rum, P.; Scartoni, V.; Costa, B.; Mazzoni, M. R.; Giusti, L. Erythro- and threo-2-hydroxynonyl substituted 2-phenyladenines and 2-phenyl-8-azaadenines: ligands for A1 adenosine receptors and adenosine deaminase. *Farmaco* **2002**, *57*, 221–233.
(54) Simon, L. N.; Bauer, R. J.; Tolman, R. L.; Robins, R. K. Calf intestine adenosine deaminase. Substrate specificity. *Biochemistry* **1970**, *9*, 573–577.
(55) Zagorowska, I.; Adamiak, R. W. 2-Aminopurine labeled RNA bulge loops. Synthesis and thermodynamics. *Biochimie* **1996**, *78*, 123–130.
(56) Schaeffer, H. J.; Thomas, H. J. Synthesis of potential anticancer agents. XV. Ribonucleosides of 2-substituted purines. *J. Am. Chem. Soc.* **1958**, *80*, 4896–4899.
(57) Peacock, H.; Maydanovych, O.; Beal, P. A. N2-Modified 2-aminopurine ribonucleosides as minor-groove-modulating adenosine replacements in duplex RNA. *Org. Lett.* **2010**, *12*, 1044–1047.
(58) Francom, P.; Robins, M. J. Nucleic Acid Related Compounds. 118. Nonaqueous Diazotization of Aminopurine Derivatives. Convenient Access to 6-Halo- and 2,6-Dihalopurine Nucleosides and 2′-Deoxynucleosides with Acyl or Silyl Halides. *J. Org. Chem.* **2003**, *68*, 666–669.
(59) Ha, S. B.; Nair, V. An improved approach to the synthesis of adenosine-5′-*N*-ethyluronamides of interest as adenosine receptor agonists. *Tetrahedron Lett.* **1996**, *37*, 1567–1570.
(60) Secrist, J. A. I.; Shortnacy-Fowler, A.; Bennett, L. L. J.; Montgomery, J. A. Synthesis and biologic evaluation of 8-substituted derivatives of nebularine (9-β-D-ribofuranosylpurine). *Nucleosides, Nucleotides* **1994**, *13*, 1017–1029.
(61) Laxer, A.; Major, D. T.; Gottlieb, H. E.; Fischer, B. (^{15}N5)-Labeled adenine derivatives: synthesis and studies of tautomerism by ^{15}N NMR spectroscopy and theoretical calculations. *J. Org. Chem.* **2001**, *66*, 5463–5481.
(62) Barton, D. H. R.; Chen, C.; Wall, M. G. Synthesis of disulfides via sulfenylation of alkyl and aryldithiopyridine *n*-oxides. *Tetrahedron* **1991**, *47*, 6127–6138.
(63) Bendich, A.; Tinker, J. F.; Brown, G. B. A synthesis of isoguanine labeled with isotopic nitrogen. *J. Am. Chem. Soc.* **1948**, *70*, 3109–3113.
(64) Tielemans, M.; Christophe, D.; Promel, R. Synthesis of 1- and 3-amino-5-*tert*-butyl-1*H*- and -3*H*-*v*-triazolo[4,5-*d*]pyrimidines as hetaryne precursors. *J. Heterocycl. Chem.* **1987**, *24*, 705–708.
(65) Montgomery, J. A.; Thomas, H. J.; Clayton, S. J. Convenient synthesis of 8-azaadenosine. *J. Heterocycl. Chem.* **1970**, *7*, 215–217.
(66) Seela, F.; Münster, I.; Lüchner, U.; Rosemeyer, H. 8-Azaadenosine and Its 2′-Deoxyribonucleoside: Synthesis and Oligonucleotide Base Pair Stability. *Helv. Chim. Acta* **1998**, *81*, 1139–1155.
(67) Vorbrüggen, H.; Krolikiewicz, K.; Bärbel, B. Nucleoside syntheses, XXII Nucleoside synthesis with trimethylsilyl triflate and perchlorate as catalysts. *Chem. Ber.* **1981**, *114*, 1234–1255.
(68) Vorbruggen, H. Some recent trends and progress in nucleoside synthesis. *Acta Biochim. Pol.* **1996**, *43*, 25–36.
(69) Niedballa, U.; Vorbrueggen, H. Synthesis of nucleosides. 9. General synthesis of *N*-glycosides. I. Synthesis of pyrimidine nucleosides. *J. Org. Chem.* **1974**, *39*, 3654–3660.
(70) Garner, P.; Ramakanth, S. A regiocontrolled synthesis of N7- and N9-guanine nucleosides. *J. Org. Chem.* **1988**, *53*, 1294–1298.
(71) Nair, V.; Chamberlain, S. D. Reductive deamination of aminopurine nucleosides. *Synthesis* **1984**, 401–403.
(72) Francom, P.; Janeba, Z.; Shibuya, S.; Robins, M. J. Nucleic acid related compounds. 116. Nonaqueous diazotization of aminopurine

nucleosides. Mechanistic considerations and efficient procedures with *tert*-butyl nitrite or sodium nitrite. *J. Org. Chem.* **2002**, *67*, 6788–6796.
(73) Sigel, H.; Brintzinger, H. Inosin-*N*(1)-oxid-Nucleotide als Komplexbildner. I. Darstellung von Inosin-*N*(1)-oxid und dessen 5prime-Monophosphat aus Adenosin-*N*(1)-oxid und dessen 5′-Monophosphat durch Desaminierung mit Nitrosylchlorid. *Helv. Chim. Acta* **1965**, *48*, 433–437.
(74) Niiya, K.; Olsson, R. A.; Thompson, R. D.; Silvia, S. K.; Ueeda, M. 2-(*N*′-alkylidenehydrazino)adenosines: potent and selective coronary vasodilators. *J. Med. Chem.* **1992**, *35*, 4557–4561.
(75) Halbfinger, E.; Major, D. T.; Ritzmann, M.; Ubl, J.; Reiser, G.; Boyer, J. L.; Harden, K. T.; Fischer, B. Molecular recognition of modified adenine nucleotides by the P2Y1-receptor. 1. A synthetic, biochemical, and NMR approach. *J. Med. Chem.* **1999**, *42*, 5325–5337.
(76) Maguire, M. H.; Sim, M. K. Adenosine deaminase. 2. Specificity and mechanism of action of bovine placental adenosine deaminase. *Eur. J. Biochem.* **1971**, *23*, 22–29.
(77) Ippel, J. H.; Wijmenga, S. S.; de Jong, R.; Heus, H. A.; Hilbers, C. W.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H. Heteronuclear scalar couplings in the bases and sugar rings of nucleic acids: their determination and application in assignment and conformational analysis. *Magn. Reson. Chem.* **1996**, *34*, S156–S176.
(78) Singh, P.; Hodgson, D. J. 8-Azaadenosine. Crystal structure of its monohydrate and conformational analysis for rotation around the glycosyl bond. *J. Am. Chem. Soc.* **1977**, *99*, 4807–4815.
(79) Altona, C.; Sundaralingam, M. Conformational analysis of the sugar ring in nucleosides and nucleotides. New description using the concept of pseudorotation. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8212.
(80) Davies, D. B.; Danyluk, S. S. Nuclear magnetic resonance studies of 5′-ribo- and deoxyribonucleotide structures in solution. *Biochemistry* **1974**, *13*, 4417–4434.
(81) Altona, C.; Sundaralingam, M. Conformational analysis of the sugar ring in nucleosides and nucleotides. Improved method for the interpretation of proton magnetic resonance coupling constants. *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344.
(82) Blackburn, B. J.; Grey, A. A.; Smith, I. C. P.; Hruska, F. E. Determination of the molecular conformation of uridine in aqueous solution by proton magnetic resonance spectroscopy. Comparison with β-pseudouridine. *Can. J. Chem.* **1970**, *48*, 2866–2870.
(83) Thibaudeau, C.; Chattopadhyaya, J. *Stereoelectronic Effects in Nucleosides and Their Structural Implications*; Uppsala University Press: Uppsala, Sweden, 1999.
(84) Ford, H. J.; Dai, F.; Mu, L.; Siddiqui, M. A.; Nicklaus, M. C.; Anderson, L.; Marquez, V. E.; Barchi, J. J. J. Adenosine deaminase prefers a distinct sugar ring conformation for binding and catalysis: kinetic and structural studies. *Biochemistry* **2000**, *39*, 2581–2592.
(85) Kim, K.; Friesner, R. A. Hydrogen Bonding between Amino Acid Backbone and Side Chain Analogues: A High-Level ab Initio Study. *J. Am. Chem. Soc.* **1997**, *119*, 12952–12961.
(86) Wilson, D. K.; Quiocho, F. A. A pre-transition-state mimic of an enzyme: X-ray structure of adenosine deaminase with bound 1-deazaadenosine and zinc-activated water. *Biochemistry* **1993**, *32*, 1689–1694.
(87) Tolman, R. L.; Robins, R. K.; Townsend, L. B. Pyrrolopyrimidine nucleosides. III. Total synthesis of toyocamycin, sangivamycin, tubercidin, and related derivatives. *J. Am. Chem. Soc.* **1969**, *91*, 2102–2108.
(88) Kurz, L. C.; Frieden, C. Adenosine deaminase converts purine riboside into an analogue of a reactive intermediate: a ^{13}C NMR and kinetic study. *Biochemistry* **1987**, *26*, 8450–8457.
(89) Jones, W.; Kurz, L. C.; Wolfenden, R. Transition-state stabilization by adenosine deaminase: 1,6-addition of water to purine ribonucleoside, the enzyme's affinity for 6-hydroxy-1,6-dihydropurine ribonucleoside, and the effective concentration of substrate water at the active site. *Biochemistry* **1989**, *28*, 1242–1247.
(90) Burnstock, G.; Fischer, B.; Hoyle, C. H. V.; Maillard, M.; Ziganshin, A. U.; Brizzolara, A. L.; von Isakovics, A.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. Structure−activity relationships for derivatives of adenosine-5-triphosphate as agonists at P2 purinoceptors: heterogeneity within P2x and P2y subtypes. *Drug Dev. Res.* **1994**, *31*, 206–219.