62. Synthesis and Conformational Analysis of 2′-Deoxy-2′-(3-methoxybenzamido)adenosine, a Rational-Designed Inhibitor of Trypanosomal Glyceraldehyde Phosphate Dehydrogenase (GAPDH)

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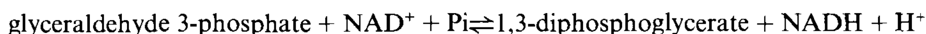
A series of 2′-benzamido-2′-deoxyadenosine analogues were synthesized in an effort to find new lead structures for the treatment of sleeping sickness. The 2′-deoxy-2′-(3-methoxybenzamido)adenosine (**1h**) was proved to be a selective inhibitor of the parasite glyceraldehyde 3-phosphate dehydrogenase which confirms the modeling studies. The solution-state conformation of 2′-(thiophene-2-carboxamido) analogue **1d** demonstrates a 2′-*endo* conformation, an orientation of the thiophene ring under the ribose moiety, and the base part occupying a '*syn*'/'*anti*' equilibrium.

1. Introduction. – Sleeping sickness is a devastating disease in many regions of subsaharan Africa. It is caused by *Trypanosoma brucei*, a protozoon belonging to the *Trypanosomatidae*. About 50 million people in 34 African countries are at high risk of getting infected with this unicellular protozoan haemoflagellate [1]. If untreated, sleeping sickness invariably leads to death.

The few drugs which are currently used to treat this disease are toxic [2] and require hospitalization [3]. Therefore, there is a current need for new effective antitrypanosoma agents. Here, we would like to present the discovery of a new lead structure by protein structure-based design, which eventually could lead to a new drug for the treatment of sleeping sickness. This approach exploits the unique features of the trypanosomal energy-generating machinery.

Trypanosomes exhibit several unusual features [4] which can be exploited for rational drug design. One of them is the fact that the bloodstream form of trypanosomes is completely dependent on glycolysis to the stage of pyruvate as the sole source of energy supply. These glycolytic reactions are sequestered in a special organelle called the glycosome [5].

Because of the crucial importance of the glycolysis in trypanosomes, most of these enzymes are potential targets for drug design. Therefore, one should exploit the structural differences that exist between the glycosomal enzymes and their human counterparts. Before starting drug design, however, basic knowledge is needed about the kinetic properties, amino-acid sequences and three-dimensional structures of the protozoan and human enzymes. Based on these studies, a molecule can be designed and synthesized which should be able to inhibit selectively the glycosomal enzymes of the trypanosomes, leaving the human isozymes unimpeded. The current work focuses on GAPDH (glyceraldehyde 3-phosphate dehydrogenase). GAPDH is a tetrameric enzyme which catalyses the conversion of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate using NAD as cofactor:



glyceraldehyde 3-phosphate + NAD^+ + Pi $\rightleftharpoons$ 1,3-diphosphoglycerate + NADH + H^+

The gene sequence of the *Trypanosoma brucei* glycosomal GADPH [6] and of the cytosol GADPH [7] was reported. The structure of glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12, gGADPH) was elucidated by X-ray crystallography [8] and compared with the structure of human-muscle GADPH (hGAPDH) for which the resolution was improved from 3.5 [9] to 2.4 Å [10]. The binding place of NAD cofactor was determined. A substantial part of this cofactor (adenosine moiety) does not directly contribute to the catalytic process, and, hence, the binding environment of this part of the molecule is not conserved between the human and trypanosomal GAPDH. Therefore, *Verlinde et al.* used this adenosine moiety of NAD as lead structure for selective inhibitor design strategy [11].

Indeed, inspection of the binding pocket of the adenosine moiety reveals significant differences between hGAPDH and gGAPDH (*Fig. 1*). Among these is the different protein backbone conformation near the adenosine ribose 2′-OH group. This part of a loop includes seven residues past the Asp (conserved in most GAPDH sequences), whose carboxylate forms two H-bonds with the secondary OH groups of the ribose part. At the third residue past this Asp, the gGAPDH backbone diverges by as much as 6.0 Å. This structural feature is not present in the human isozyme, where the GAPDH backbone stays close to the ribose ring. This difference is probably caused by the fact that in gGAPDH, the first amino acid past the Asp is a methionine (whose N forms a H-bond with the side chain of the conserved Asp), while in human GAPDH it is a proline (containing a N that lacks H-bond properties). As a consequence, the Ψ angle of the Asp changes from −171° in gGAPDH to 105° in human GAPDH, ultimately leading to a different loop conformation. Therefore, only gGAPDH exhibits a cleft that reaches from the ribosyl OH groups to the side chain of Asn 39. Due to the presence of Met 38 and Val 205 (of another subunit of the GAPDH tetramer) on both sides, this cleft is largely hydrophobic. As the ribosyl 2′-OH group projects right into this hydrophobic cleft, it seemed a promising starting point for making derivatives that would occupy this area which, in human GAPDH, is largely occupied by the side chain of Ile 37. Because 2′-OH derivatives in the form of esters or ethers would lead to the loss of the H-bond with the proximate Asp 37, we replaced the 2′-OH group by an amino function, which, upon amidation, would then retain H-bond donor properties. Force-field calculation confirmed this view and indicated that a good-quality H-bond to Asp 37 is conserved, and that eventual aromatic substituents at the *C*=O of the amide would fit perfectly in the

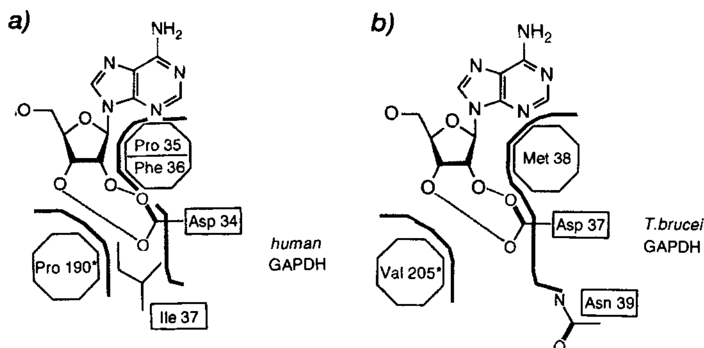
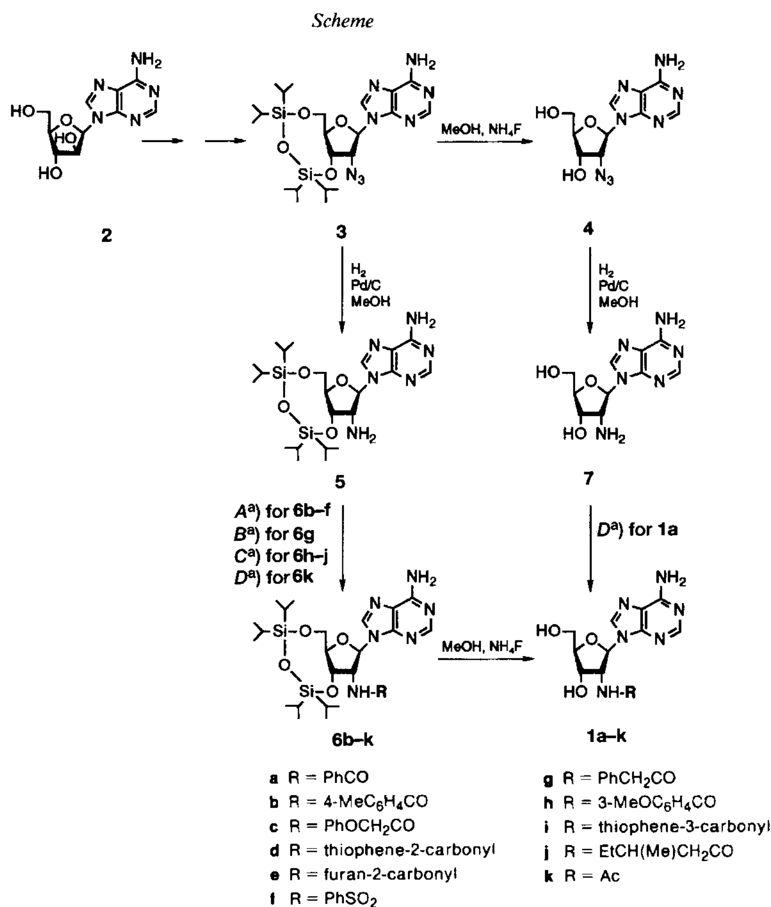


Fig. 1. *Schematic presentation of the binding mode of the ribose moiety of NAD to* a) *human GAPDH and* b) T. brucei *GAPDH, showing the residues that differ*

Scheme



a) *Method A*: RCOCl, CH_2Cl_2, Py. *Method B*: RCOCl, Py, $Me_2NC_5H_4N$. *Method C*: RCOOH, DMF, DCC, *N*-hydroxysuccinimide. *Method D*: $(RCO)_2O$, CH_2Cl_2, Py.

selectivity cleft. This led to the modeling of the 2′-benzamide compound **1a** and some derivatives. Modeling also suggested that H-bond acceptors in *meta* position of the aromatic substituent could reinforce the interaction with the present Asn 39 of gGAPDH. A MeO substituent (in compound **1h**) appeared to meet this modelling criterion.

Here, we describe the synthesis and structure-activity relationship of 2′-amido-substituted 2′-deoxyadenosines. Based on NMR data, their conformation in solution state was examined. Some of these molecules are new lead compounds for the development of selective anti-trypanosomal drugs.

2. Chemistry. – The 2′-modified nucleosides were synthesized starting from 9-(β-D-arabinofuranosyl)adenine (*Scheme*). The 3′-OH and the 5′-OH group of 9-(β-D-arabinofuranosyl)adenine (**2**) were simultaneously protected using *Markiewicz* protecting group [12], yielding 80% of 9-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]adenine. The N_3 group was introduced in the 2′-position using trifluoromethanesulfonyl chloride and NaN_3 (→ **3** in 68% yield) [13]. Deprotection with NH_4F/MeOH yielded **4**. The N_3 function of **3** was reduced with H_2 in MeOH in the presence of Pd/C without loss of the $O[(i\text{-}Pr)_2Si]_2$ protecting group. This step seemed to be influenced by the choice of solvent, and substantially lower yields of **5** were obtained using CH_2Cl_2 or

Table 1. *¹H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *for the* $O[(i\text{-}Pr)_2Si]_2$*-Protected Nucleoside Derivatives* **5** *and* **6b–k** *in* $CDCl_3$

	H–C(2)	H–C(8)	NH_2	H–C(1′)	H–C(2′)	H–C(3′)	H–C(4′)	H_A–C(5′)
5	8.08	8.26	7.28	5.72	*ca.* 3.97[a])	4.82	*ca.* 3.97[a])	*ca.* 3.97[a])
6b	8.04	8.22	6.00	6.12	4.92	5.55	*ca.* 4.10[a])	*ca.* 4.10[a])
c	7.99	8.23	5.87	6.03	4.83	5.39	*ca.* 4.05[a])	*ca.* 4.05[a])
d	8.01	8.19	6.19	6.12	4.93	5.55	*ca.* 4.09[a])	*ca.* 4.09[a])
e	8.02	8.21	6.14	6.10	4.94	5.47	*ca.* 4.08[a])	*ca.* 4.08[a])
f	7.89	8.16	5.83	6.13	4.24	5.26	*ca.* 4.00[a])	*ca.* 4.00[a])
g	7.94	8.18	6.02	5.95	4.67	5.32	3.84	*ca.* 3.99[a])
h	8.04	8.26	5.77	6.11	4.90	5.54	*ca.* 4.11[a])	*ca.* 4.11[a])
i	8.02	8.23	5.83	6.10	4.88	5.49	*ca.* 4.10[a])	*ca.* 4.10[a])
j	7.97	8.24	5.78	5.95	4.73	5.38	*ca.* 4.14[a])	*ca.* 4.14[a])
k	7.97	8.24	5.81	5.96	4.76	5.31	*ca.* 4.04[a])	*ca.* 4.04[a])

	H_B–C(5′)	NH–C(2′)	i-Pr	*J*(1′,2′)	*J*(2′,3′)	*J*(2′,NH)	*J*(3′,4′)
5	*ca.* 3.97[a])	1.96	1.0 –1.1	3.6	6.3	[b])	6.3
6b	*ca.* 4.10[a])	7.25	1.0 –1.25	3.2	7.2	4.1	6.3
c	*ca.* 4.05[a])	7.67	0.95–1.2	3.7	7.6	4.5	6.3
d	*ca.* 4.09[a])	7.12	1.0 –1.25	3.2	7.6	3.7	5.9
e	*ca.* 4.08[a])	7.11	1.0 –1.2	3.6	8.9	3.5	4.6
f	*ca.* 4.00[a])	5.58	0.95–1.2	2.7	7.4	2.4	6.9
g	*ca.* 3.99[a])	6.46	0.85–1.15	3.3	7.6	4.0	6.9
h	*ca.* 4.11[a])	7.36	1.0 –1.2	3.3	7.3	3.6	6.8
i	*ca.* 4.10[a])	7.06	1.0 –1.2	[b])	[b])	[b])	[b])
j	*ca.* 4.14[a])	6.46	1.05–1.2	3.6	7.6	4.1	6.0
k	*ca.* 4.04[a])	6.44	1.0 –1.15	4.0	7.7	4.7	5.5

[a]) Due to overlap of this *ABC* part of the *ABCX* spin system, no exact assignment of these signals could be done.
[b]) Not determined.

AcOEt instead of MeOH. Acylation of the aliphatic NH_2 group could be carried out without acylation of the adenosine moiety as side reaction. Four different procedures were used. When the acyl chloride was commercially available, *Method A* (acyl chloride or sulfonyl chloride, CH_2Cl_2, pyridine) or *Method B* (acyl chloride, pyridine, 4-(dimethylamino)pyridine) was used. Otherwise, the carboxylic acid was activated with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide [14] (*Method C*) or by converting it into the anhydride (*Method D*). All these procedures gave satisfactory yields (60–90%) of **6b–k**. Finally the protecting group was removed by treatment with NH_4F in MeOH at 60° [15] [16] yielding **1b–k**. The 2′-benzamido-2′-deoxyadenosine (**1a**) was synthesized by reaction of unprotected 2′-amino-2′-deoxyadenosine (**7**; obtained from **4** by reduction) with benzoic anhydride in a mixture of CH_2Cl_2 and pyridine. All final compounds **1a–k** were identified by ^1H-NMR, ^{13}C-NMR, UV, and elemental analysis.

3. NMR Parameters and Conformational Analysis. – The ^1H-NMR parameters for the compounds **5**, **6b–k**, and **1a–k** are given in *Tables 1* and *2* and the ^{13}C-NMR parameters in *Table 3*. Since the conformational features are derived from the NMR parameters, efforts were made to obtain the assignment of the resonances with great certainty.

Assignment of the Resonances and Extraction of the Coupling Constants. The resonances for the ribose moiety were first assigned by ^1H,^1H-COSY 45 experiments and by considering the reciprocity of the vicinal coupling constants. By way of illustration we give the ^1H,^1H-COSY-45 spectrum of compound **1d** in (D_6)DMSO solution (see *Fig. 2*).

The substitution site on C(2″) or C(3″) of the furan or thiophene ring was verified using the vicinal and the long-range coupling constants, by comparison with the data given in [17].

Conformational Analysis of the Exocyclic C(4′)–C(5′) Bond. We followed the method proposed by *Haasnoot et al.* [18]. It is accepted that the solution conformation of the $CH_2(5')OH$ moiety relative to the furanose ring may comprise three types of rotamers (see **A–C**) which are denoted g^+, g^-, and t (delineating the conformation of the backbone fragment C(3′)–C(4′)–C(5′)–O(5′)). The mole fractions of each rotamer are calculated from *Eqns. 1–3*,

$$x_{g^+} + x_{g^-} + x_t = 1 \qquad (1)$$

$$2.4x_{g^+} + 10.6x_{g^-} + 2.6x_t \approx 3.8 \text{ Hz for } J(4',5'A) \qquad (2)$$

$$1.3x_{g^+} + 3.8x_{g^-} + 10.5x_t \approx 3.3 \text{ Hz for } J(4',5'B) \qquad (3)$$

with x_{g^+}, x_{g^-}, and x_t being the mole fractions of each rotamer and with the theoretical values for the coupling constants proposed by *Haasnoot* for $J(4',5'A)$ and $J(4',5'B)$ for each rotamer.

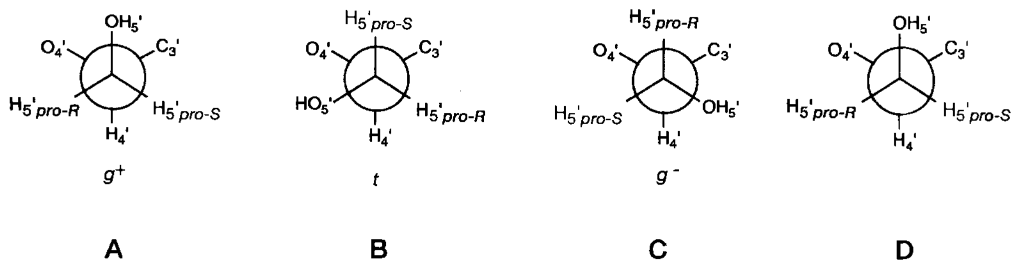


Table 2. *^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *for the Nucleoside Derivatives* **1a–k** *in (D_6)DMSO*

	H–C(2)	H–C(8)	NH_2	H–C(1′)	H–C(2′)	H–C(3′)	OH–C(3′)	H–C(4′)	H_A–C(5′)	H_B–C(5′)	OH–C(5′)	NH–C(2′)
1a	8.15	8.27	7.39	6.23	5.33	4.36	5.77	4.11	3.74	3.63	5.68	8.38
b	8.14	8.27	7.37	6.22	5.32	4.35	5.75	4.11	3.74	3.63	5.67	8.25
c	8.14	8.28	7.37	6.04	5.14	4.28	5.98	4.08	3.71	3.61	5.64	8.05
d	8.15	8.25	7.4	6.19	5.29	4.32	5.78	4.09	3.71	3.61	5.60	8.43
e	8.14	8.27	7.38	6.17	5.26	4.32	5.89	4.10	3.73	3.61	5.68	8.08
f	8.01	8.09	7.27	5.84	4.65	4.08	5.84	3.98	3.62	3.51	5.68	*ca.* 8.2[b])
g	8.14	8.27	7.36	5.99	5.07	4.23	5.82	4.06	3.69	3.59	5.57	8.23
h	8.15	8.27	7.38	6.22	5.33	4.35	5.75	4.11	3.74	3.62	5.67	8.37
i	8.14	8.26	7.38	6.18	5.31	4.32	5.77	4.10	3.73	3.62	5.65	8.24
j	8.12	8.24	7.32	5.94	5.09	4.19	5.75	4.03	3.67	3.59	5.57	7.87
k	8.14	8.26	7.36	5.93	5.08	4.22	5.70	4.03	3.67	3.57	5.56	8.00

	J(1′,2′)	J(2′,3′)	J(2′, NH)	J(3′,4′)	J(3′, OH–C(3′))	J(4′,5′A)	J(4′,5′B)	J(5′A, OH–C(5′))	J(5′B, OH–C(5′))	J(5′A,5′B)
1a	8.6	5.2	8.3	[b])	[b])	*ca.* 3.5	3.3	4.1	7.4	−12.1
b	8.5	5.4	*ca.* 9.0[a])	*ca.* 1[b])	4.1	*ca.* 3.5	3.3	4.0	7.7	−11.4
c	8.4	5.4	8.6	*ca.* 1[b])	[b])	[b])	[b])	[b])	[b])	[b])
d	8.5	5.4	8.5	[b])	4.1	3.9	3.3	4.1	7.2	−12.1
e	8.5	5.4	8.5	[b])	3.6	3.9	3.3	4.0	7.6	−11.7
f	8.7	4.9	[b])	*ca.* 1[b])	[b])	3.8	3.0	3.8	8.0	−11.8
g	8.5	5.3	8.6	*ca.* 1[b])	4.3	3.9	3.3	4.4	7.3	−12.1
h	8.6	5.3	8.3	1.0	4.3	3.9	3.3	3.9	7.6	−11.8
i	8.6	5.3	8.6	[b])	3.2	3.9	3.3	4.2	7.4	−11.7
j	8.6	5.4	8.8	*ca.* 1[b])	[b])	[b])	[b])	[b])	[b])	[b])
k	8.6	5.2	8.6	*ca.* 1[b])	4.1	4.1	3.4	4.2	7.3	−11.9

[a]) Overlap with H–C(8). [b]) Not resolved.

The experimental values that were introduced for these coupling constants were the averaged values of all compounds **1** gathered in *Table 2*, where these values could be extracted on a first-order basis. The cases where the resolution was not good enough to estimate the value of the coupling constants within a range of ±0.3 Hz were not considered (*e.g.* **1a** and **1b**).

It is possible to assign H_A–C(5′) to $H_{pro\text{-}R}$–C(5′) from the study of the 2D NOESY experiment (see below). Unfortunately, this assignment can not be verified by a criterion proposed by *Remin* and *Shugar* [19] which states that when two conditions are fulfilled, namely $\delta(H_A\text{–C(5′)}) > \delta(H_B\text{–C(5′)})$ and $J(4',5'A) < J(4',5'B)$, the most shielded proton (H_B–C(5′)) is $H_{pro\text{-}S}$–C(5′). Unfortunately, for our products the pattern of the protons at the lowest field (H_A–C(5′)) has a larger coupling constant with H–C(4′) than its geminal partner, so that the conditions of [19] to assign $H_{pro\text{-}S}$–C(5′) were not fulfilled.

We calculated the mole fractions. The values of *Set 1* with $x_{g^+} = 0.69$, $x_{g^-} = 0.17$, and $x_t = 0.14$ hold for the situation in which H_A–C(5′) is $H_{pro\text{-}R}$–C(5′) and those of *Set 2* with $x_{g^+} = 0.67$, $x_{g^-} = 0.07$, and $x_t = 0.25$ for that in which H_A–C(5′) is $H_{pro\text{-}S}$–C(5′). Although only *Set 1* must be considered, it seems that for both possibilities, conformation g^+, *i.e.* that with OH–C(5′) above the ring, is more populated than the conformations of t and g^- taken together. This information agrees with the known data [20], namely that domination of the g^+ orientation in purine nucleosides is expected for both a C(3′)-*endo* or C(2′)-*endo* conformation in the furanose ring.

Table 3. *^{13}C-NMR Data of the Common Sugar and Base Moiety*

	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	CO	i-Pr
5	152.50	149.08	119.31	156.14	139.73	89.12	56.98	71.37	82.50	62.50	–	12.20–17.39
6b	152.70	149.35	120.23	155.55	140.33	88.35	56.64	70.96	83.68	63.11	167.80	12.24–17.35
c	152.80	149.47	120.14	155.45	140.11	87.83	55.62	70.54	83.74	62.83	169.37	12.58–17.33
d	152.66	149.24	120.14	155.60	140.23	88.21	56.66	70.89	83.66	63.12	162.43	12.23–17.29
e	152.71	149.37	120.14	155.63	140.15	88.02	55.92	70.98	83.81	63.23	158.93	12.26–17.34
f	152.69	149.10	120.32	155.39	140.42	89.34	58.16	69.60	82.93	61.98	–	12.37–17.24
g	152.69	149.30	120.12	155.54	140.15	88.16	56.25	70.31	83.50	62.67	171.98	12.18–17.32
h	152.73	149.44	120.30	155.42	140.36	88.33	56.67	70.85	83.70	63.01	167.69	12.67–17.34
i	152.73	149.40	120.11	155.59	140.03	88.01	56.40	70.96	83.81	63.22	163.47	12.28–17.33
j	152.66	149.43	120.18	155.41	140.17	88.20	56.08	70.63	83.73	63.04	173.47	12.59–17.32
k	152.77	149.49	120.12	155.49	139.94	87.74	55.94	70.62	83.88	63.09	170.76	12.55–17.32
1a	152.43	149.21	119.54	156.26	140.02	86.06	55.53	70.59	87.52	62.14	166.63	
b	152.49	149.24	119.59	156.31	140.10	86.12	55.50	70.65	87.59	62.19	166.53	
c	152.44	149.30	119.38	156.23	139.81	86.34	54.60	70.69	87.57	62.02	168.01	
d	152.48	149.24	119.48	156.25	139.94	85.76	55.31	70.52	87.48	62.06	161.35	
e	152.44	149.22	119.53	156.29	140.08	86.27	54.62	70.63	87.53	62.10	157.69	
f	151.86	148.47	119.65	156.16	139.88	87.42	57.93	71.07	85.73	62.02	–	
g	152.44	149.38	119.27	156.17	139.68	86.14	55.13	70.66	87.59	62.01	170.61	
h	152.45	149.23	119.56	156.28	140.01	86.05	55.57	70.61	87.54	62.14	166.42	
i	152.46	149.25	119.50	156.25	139.95	85.89	55.11	70.58	87.50	62.10	162.23	
j	152.36	149.37	119.27	156.14	139.69	86.06	54.78	70.67	87.60	62.04	172.08	
k	152.48	149.37	119.38	156.23	139.87	86.03	54.89	70.57	87.47	62.06	169.76	

The Conformation of the Ribofuranose Ring. The torsion angles $\theta(1',2')$, $\theta(2',3')$, and $\theta(3',4')$, *i.e.* the torsion angle between the protons on C(1′), C(2′), C(3′), and C(4′), were estimated using a *Karplus* equation as modified by *Haasnoot* [21] [22] (see *Eqn. 4*).

$$^{3}J(\mathrm{H,H}) = 13.7\cos^2\Phi - 0.73\cos\Phi + \Sigma_i\Delta\chi_i\,[0.56 - 2.47\cos^2(z_i\varphi + 16.9\,|\Delta\chi_i|)] \quad (4)$$

A straightforward comparison with published data for β-D-ribofuranoses cannot be applied, since in the present case corrections must be introduced for the N-substituent at C(2′). In *Eqn. 4*, Φ denotes the H,H torsion angle, and the third term is a correction factor for the electronegativity and orientation of the substituents. Σ stands for the sum of the effects of all substituents of the vicinal C-atoms. $\Delta\chi_i$ is the difference in *Huggins* electronegativity between the H-atom and the substituent and is given by *Eqn. 5*.

$$\Delta\chi_i^{\mathrm{subst}} = \Delta\chi^{\alpha} - 0.19\,\Sigma\Delta\chi^{\beta} \quad (5)$$

For O, $\Delta\chi$ is 1.3, for C 0.4, and for N 0.85. z_i is determined by the orientation of the substituents geminal to the protons whose coupling constant is considered. The terms 13.7, 0.73, 0.56, 2.47, and 16.9 in *Eqn. 4* were obtained from the coupling constants of a large set of compounds.

We calculated Φ for the range values found for the corresponding coupling constants measured for the compounds **1** gathered in *Table 2*. The coupling constants $J(1',2')$, $J(2',3')$, and $J(3',4')$ point to a conformation which in the pseudorotation cycle is in the range of the C(2′)-*endo* conformation (or in the neighborhood of ^{2}E) [23].

Following *Altona* and *Sundaralingam* [24], the conformation of a five-membered ring can be given by two parameters, the 'phase angle' of pseudorotation P and the degree of

pucker Φ_m. A plot of the vicinal coupling constants *vs.* the torsion angle as derived from the modified *Karplus* equation gave four torsion angles for each ^1J(H,H) value (0–360°), although only one value is realistic. To obtain a more precise information about the conformation of our compounds, the data were introduced in a model structure and by optimizing it through energy minimization (using the AMBER force yield in the 'Hyper Chem. Release 2' computer programme) with constraints on $\Phi_{H,H}$. One set is found: $P = 150°$, $\Phi_m = 37.2°$, in the range of the $^2T_1$ to ^2E conformations. *De Leeuw* and *Altona* [25] found for 2′-amino-2′-deoxyribose with values $J(1′,2′) = 8.41$, $J(2′,3′) = 5.57$, and $J(3′,4′) = 1.30$ Hz a $P = 162°$ and $\Phi_m = 35°$, indeed a set of values very close to the set extracted by us, although these data are not always evident.

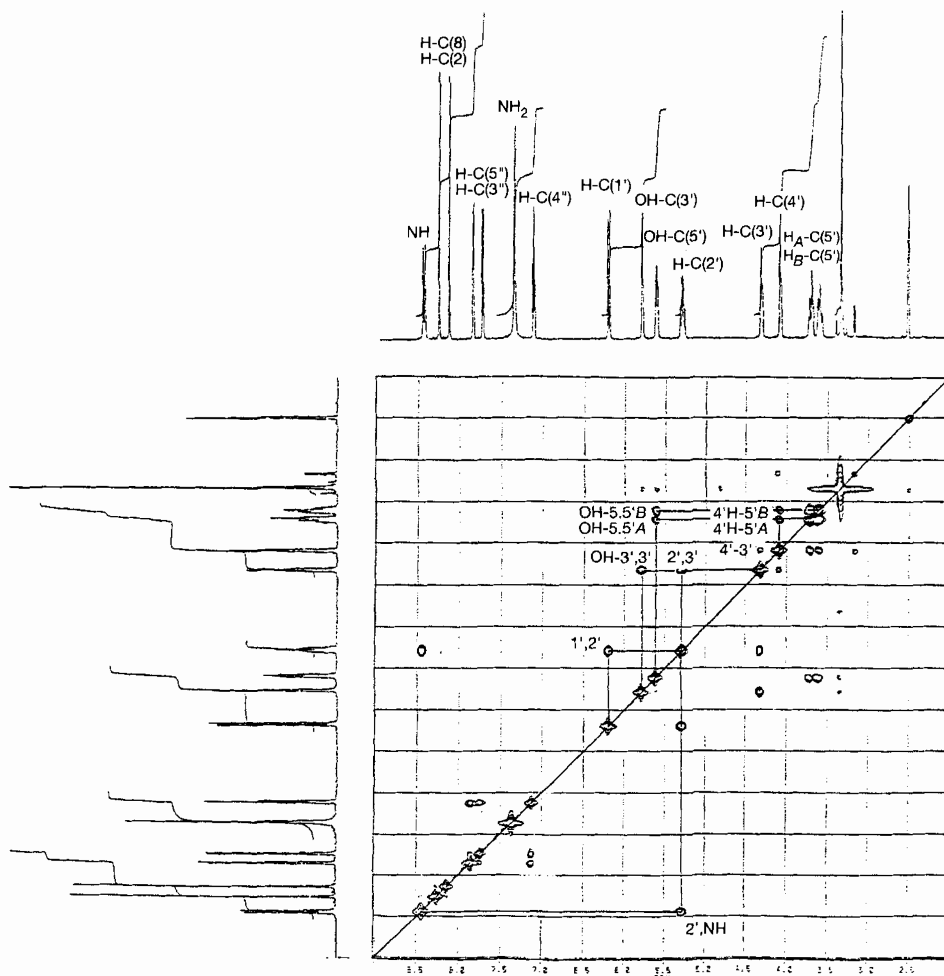


Fig. 2. *^1H,^1H-COSY-45 Spectrum of compound* **1d** *in (D_6)DMSO*. The connectivities between H–C(1′), H–C(2′), NH–C(2′), H–C(3′), OH–C(3′), and H–C(4′) and the cross-peaks of H–C(4′) and OH–C(5′) with H_A–C(5′) and H_B–C(5′) are indicated by a full line.

Indeed, it was found [26] that for 2′-substituted adenosine derivatives, the amount of the C(3′)-*endo* conformation increases with the electronegativity of the 2′-substituent (consequently, because of the low electronegativity of the N-atom, a more important proportion of C(2′)-*endo* conformation is expected when OH–C(3′) is axial). It was even concluded that the *gauche* effect plays an important role in the most preferred sugar conformation, because this effect directs the torsion angles of X–C–C–Y (X, Y = electronegative substituents) to g^+ or g^- values and thus tends to avoid an antiperiplanar position. In ribose, many such groupings are found, favoring the C(2′)-*endo* conformation [27] [28]. Deviations were found in the crystal structure of β-D-2′-amino-2′-deoxyadenosine [29]. Here, a C(2′)–NH···O–C(3′) H-bonding was found, not observed in other derivatives, leading to an *O*–C(4′)-*endo* puckering. The *O*–C(4′)-*endo* conformation is on the left side of the pseudorotation cycle. From our measurements, we can safely say that our compounds in (D_6)DMSO solution do not display a H-bonding between the amido N-atom on C(2′) and OH–C(3′).

The Conformation of the Glycosidic Bond. The conformation of the glycosidic bond (the torsion angle O–C(4′)–C(1′)–N(9)–C(4)) was estimated from a 2D-NOESY experiment with compound **1d**. The resonances for H–C(3″), H–C(4″), and H–C(5″) of the thiophene moiety were assigned by considering the coupling constants given in [17].

With a mixing time of 50 ms, we found a NOE, between H–C(1′) and H–C(2) with a mixing time of 80 ms, NOE's between H–C(1′) and H–C(3″) as well as H–C(4″) were observed. This suggests that the plane of the thiophene ring is situated below the ring. This finding was verified by the absence of NOE's between the substituent and H–C(2′), but there were NOE's between H–C(4′) and H–C(4″) and H–C(5″).

There was clearly a NOE between H–C(3′) and H_A–C(5′). Herefrom we can derive that H_A–C(5′) is the $H_{pro\text{-}R}$–C(5′) (see **D**).

With a mixing time of 80 ms, the NOE's H–C(3″)/H–C(2), as well as H–C(8)/H_A–C(5′), and H–C(2)/NH–C(2′) were observed, suggesting an '*anti*' conformation (see *Fig. 3*). At the same time, there was also a NOE between H–C(2) and H_A–C(5′) as well as between H–C(8) and H–C(5″) and NH–C(2′), suggesting a '*syn*'-conformation. The contacts between H–C(3′) and H–C(3″) and H–C(4″) can only be due to diffusion effects over H–C(4′). These cross-peaks were observed in the spectrum. These data do not point out to a straightforward conformation about the glycosidic bond, but some data agree with a '*syn*' and other with an '*anti*' conformation. It is known that, in crystal structures, purine nucleosides with a C(2′)-*endo* conformation of the ribose ring adopt the '*anti*'- and '*syn*'-conformation in nearly equal distribution [30]. This equilibrium also occurs in solution and is supported by NMR studies [31].

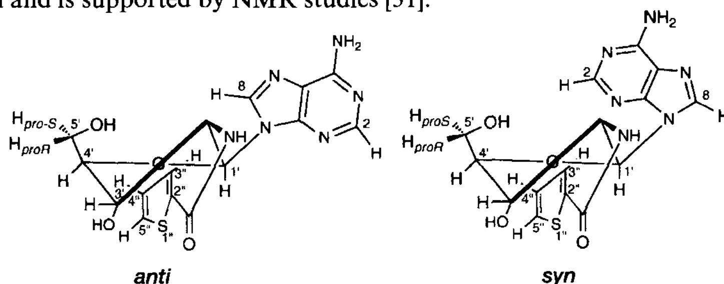


Fig. 3. 'anti'- *and* 'syn'-*Conformation of the base relative to the sugar moiety in compound* **1d**

Also a NOE between both protons on C(5′) and H–C(1′), the protons of the thiophene ring, and the amide proton can only be explained by diffusion effects *via* H–C(4′). The NOE between H–C(1′) and H_A–C(5′) may be explained by a diffusion effect over H–C(2′) (the respective cross-peaks H–C(1′)/H–C(2′) and H–C(2′)/H_A–C(5′) and H_B–C(5′) are observed).

Special Study of **1j**. Because of a chiral environment, we should obtain a different ^{1}H-NMR spectrum for the amido moiety of the two diastereoisomers **I** and **II** of **1j**, a phenomenon usually encountered in amino acids [32]. Because of the collapse of some signals, we tried to differentiate them by a COSY-45 experiment. But the H–C(3″) signal of **I** and **II** nearly collapsed, allowing only a partial differentiation between the two proton systems (see *Table 4*).

The ^{13}C-NMR Spectra. The resonances in the ^{1}H-noise-decoupled ^{13}C-NMR spectra were first assigned by comparison with the data given in [33]. These assignments were verified by a ^{13}C,^{1}H heteronuclear correlated experiment, where the ^{13}C-NMR resonances were assigned from the knowledge of the ^{1}H-NMR resonances. Compound **1h** was completely analyzed in this way. Herefrom, there is no doubt that although there is a small difference between the resonances of C(1′) and C(4′), the latter is found at a higher frequency than the former. The low resonance position for C(2′) at δ 55 is an indication that there is a N-substitution on C(2′). The resonance for C(5′) is found at a lower frequency than the resonance for C(3′).

For the resonances of the protonated C-atoms of the adenine moiety of **1h**, it is clear that the ^{1}H-NMR resonance at the lowest frequency is linked to the C-atom which resonates at the highest frequency in the ^{13}C-NMR spectrum. The other ^{13}C-NMR resonances for adenine were assigned by comparison with known data [33]. All other ^{13}C-NMR spectra were interpreted by analogy with that of **1h**.

4. Biological Results. – *Table 5* shows the I_{50} values (concentrations at which the enzym remains 50% active, determined as in [11]) of compounds **1a–k**. These inhibition studies pointed out that the designed 2′-benzamido-2′-deoxyadenosine (**1a**) is a good lead structure for selective gGAPDH inhibition. It showed an I_{50} value of 3.3 mM for glycosomal *Leishmania mexicana* GAPDH compared to 50 mM for adenosine. Note that the easier available *L. mexicana* and *Trypanosoma brucei* glycosomal GAPDH are identical in sequence in the region of interest.

Similar I_{50} values were observed for compounds **1b**, **1d**, and **1i** (2.6, 2.4, and 3.3 mM, resp.). In contrast, compound **1e** did not show any activity. These results are a clear example of an isosteric replacement of a benzene ring by a thiophene moiety. The furan

Table 4. *^{1}H-NMR Data (δ [ppm], J [Hz]) for Both Diastereoisomers* **I** *and* **II** *of Compound* **1j**[a])

	H_A–C(2″)	H_B–C(2″)	H–C(3″)	Me–C(3″)	H_A–C(4″)	H_B–C(4″)	Me(5″)
I	2.03	1.88	1.56	0.67[b])	1.14[b])	0.99[b])	0.72[b])
II	1.99	1.84	1.56	0.54[b])	0.99[b])	0.91[b])	0.63[b])

	$J(2''_A,3'')$	$J(2''_B,3'')$	$J(2''_A,2''_B)$	$J(3''$,Me–C(3″))	$J(4''_A,4''_B)$	$J(4''_A,5'') \approx J(4''_B,5'')$
I	7.3	8.5	−12.5	6.7	−12.5	7.3
II	7.3	8.5	−12.5	6.6	−12.5	7.3

[a]) Numbering of the amido moiety: Me(5″)CH_2(4″)CH(3″)(Me)CH_2(2″)CO(1″)N.
[b]) These shifts may be reversed for **I** and **II**.

Table 5. *GAPDH Inhibition:* I_{50} *Values* [mM]

	Adenosine	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**	**1k**
G.L.m. GAPDH[a])	50	3.3	2.6	> 10	2.4	> 10	> 10	> 10	0.3	3.3	> 20	> 20
R.m. GAPDH[b])	10	4 (90%[c]))	4 (90%[c]))	[d])	30	[d])	[d])	[d])	30	[d])	[d])	[d])

[a]) G.L.m. = glycosomal *Leishmania mexicana.* [b]) R.m. = rabbit muscle. [c]) Remaining activity [%] at given concentration. [d]) Not determined.

ring can not be considered as a benzene isoster. The highest inhibition was observed with 2′-deoxy-2′-(3-methoxybenzamido)adenosine (**1h**), whose I_{50} of 0.3 mM was 170 times better than that of adenosine. This result is also in agreement with the modeling predictions [11]. The commercially available rabbit-muscle GAPDH (probably very close to the human one) was used to evaluate the compounds on selectivity, and enzyme kinetics proved that all these compounds are highly selective (rabbit-muscle GAPDH still shows more than 90% activity at 4 mM). Compound **1h**, *e.g.* inhibited the rabbit muscle GAPDH 3 times less than adenosine, resulting in a 510-fold gain in selectivity.

Chain prolongation between the amido function and the aromatic moiety (**1c**, **1g**) or replacing the aromatic ring by an aliphatic chain (**1j**, **1k**) decreased the activity. Also, the replacement of the amido function by a sulfonamido function (**1f**) gave a less active compound.

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Experimental Part

General. Anh. solvents were obtained as follows: pyridine was refluxed overnight in the presence of KOH and distilled; CH_2Cl_2 was obtained by distillation after reflux overnight with CaH_2; H_2O was removed from 99% *N,N*-dimethylformamide (DMF) (*Janssen*) by storing on *Linde* type-4-Å molecular sieves, followed by distillation under reduced pressure. TLC: precoated *Merck* silica gel F_{254} plates; detection with UV light at 254 nm and sulfuric acid/anisaldehyde spray. Column chromatography: *SÜD-Chemie* silica gel (0.2–0.05 mm). M.p.: in capillary tubes; electrothermal (*IA 9000* series) digital melting point apparatus; uncorrected. UV Spectra in MeOH; *Perkin-Elmer* UV/VIS spectrophotometer; λ_{max} in nm (lg ε). NMR Spectra: *Bruker-WH-360* spectrometer; ^{1}H: at 360.136 MHz, pulse angle 19° (2 μs), resolution 0.37 Hz/point; ^{13}C: at 90.556 MHz, pulse width 18°, no relaxation delay, resolution 1.327 Hz/point. Solns. of *ca.* 30 mg compound in 0.6 ml of (D_6)DMSO (for **1a–k**) or $CDCl_3$ (for **5** and **6b–k**) at 19°; the solvent signals were used as secondary references (^{1}H: (D_6)DMSO (= 2.50 ppm) and $CDCl_3$ (= 7.23 ppm) *vs.* Me_4Si; ^{13}C: (D_6)DMSO (= 39.60 ppm) or $CDCl_3$ (= 76.89 ppm) *vs.* Me_4Si). COSY 45 Spectrum: absolute-value mode using a 90°(^{1}H)-t_1-90°(^{1}H)-t_2 sequence [34]; a 0.5 × 1 K data matrix was obtained with 16 scans; no zero-filling, but resolution enhancement in κ_1 and κ_2 by a $\pi/2$-shifted sine-bell function in t_1 and t_2; 90°(^{1}H) pulse of 5.0 μs. ^{13}C,^{1}H Heteronuclear correlated experiment: *Bax-Morris* sequence [35], *i.e.* 90°(^{1}H)-t_1/2–180°(^{13}C)-t_1/2-Δ_1-90°(^{1}H)-90°(^{13}C)-Δ_2-t_2; 90°(^{1}H) pulse of 8.2 μs, 90°(^{13}C) pulse of 10.2 μs; Δ_1 optimized to 3.2 ms and Δ_2 to 2.1 ms; relaxation delay of 1 s; a 1 × 4 K matrix was obtained using 88 scans. 2D-NOESY Experiment: sequence proposed by *Macura et al.* [36], *i.e.* 90°(^{1}H)-t_1-90°(^{1}H)-τ_m-χt_1-90°(^{1}H)-t_2; relaxation delay of 1.2 s, 90°(^{1}H) pulse of 5 μs, mixing times τ_m of 50 and 80 ms randomly varied by 10% to suppress the zero quantum *J* cross-peaks; a matrix of 0.5 · 1 K data points was obtained using 16 scans, zero-filled in the *F*1 direction; 45°-shifted sine-bell function in each direction. Electron impact (EI) and chemical ionization (CI) MS: *Kratos-concept-1H* mass spectrometer. Elemental analyses were performed at the University of Konstanz, Germany.

1. *2′-Amino-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**5**). A soln. of 2′-azido-2′-deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**3**; 3.00 g, 5.61 mmol) in MeOH (60 ml) was hydrogenated at r.t. and at $8 \cdot 10^{-2}$ N/m^2 pressure in the presence of 10% Pd/C (500 mg) for 15 h. The mixture was filtered and evaporated to yield 2.46 g (86%) of **5**. UV (MeOH): 260 (4.15). MS: 508.2650 (M^+, $C_{22}H_{40}N_6O_4Si_2^+$, calc. 508.2647).

2. *2′-Deoxy-2′-(4-methylbenzamido)-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6b**). At 0°, 4-toluoyl chloride (0.21 ml, 245 mg, 1.59 mmol) was added to **5** (663 mg, 1.3 mmol) in CH_2Cl_2 (10 ml) and pyridine (3 ml). The soln. was stirred for 2 h at r.t., evaporated, and purified by CC: 614 mg (75%) of **6b**. White solid. UV (MeOH): 250 (4.33). ^{1}H-NMR ($CDCl_3$): 2.40 (Me); 7.25 (2 H_m); 7.67 (2 H_o). ^{13}C-NMR ($CDCl_3$): 21.34 (Me); 126.76 (2 C_o); 129.30 (2 C_m); 130.46 (C_{ipso}); 142.51 (C_p). MS: 626.3068 (M^+, $C_{30}H_{46}N_6O_5Si_2^+$, calc. 626.3112).

3. *2′-Deoxy-2′-(2-phenoxyacetamido)-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6c**). A soln. of **5** (712 mg, 1.40 mmol) in CH_2Cl_2 (10 ml) and pyridine (3 ml) was cooled to 0° and phenoxyacetyl chloride (0.23 ml, 2.84 mg, 1.67 mmol) added. After stirring for 2 h at r.t., TLC (CH_2Cl_2/MeOH 95:5) revealed incomplete reaction, and an additional amount (0.1 ml) of phenoxyacetyl chloride was added. After 1 h, the mixture was evaporated and purified by CC (AcOEt): 560 mg (62%) of **6c**. UV (MeOH): 259 (4.18). ^{1}H-NMR ($CDCl_3$): 4.47 *A* of *AB* ($J(A,B) = -15.1$, 1 H, CH_2); 4.56 (*B* of *AB*, 1 H, CH_2); 6.88 (2 H_o); 7.02 (H_p); 7.30 (2 H_m). ^{13}C-NMR ($CDCl_3$): 67.21 (CH_2CO); 114.38 (2 C_o); 122.12 (C_p); 129.70 (2 C_m); 156.97 (C_{ipso}). MS: 642.3017 (M^+, $C_{30}H_{46}N_6O_6Si_2^+$, calc. 642.3066).

4. *2′-Deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-(thiophene-2-carboxamido)adenosine* (**6d**). To a cooled (ice-bath) soln. of **5** (830 mg, 1.63 mmol) in CH_2Cl_2 (10 ml) and pyridine (3 ml) thiophene-2-carbonyl chloride (0.225 ml, 309 mg, 2.11 mmol) was added dropwise. After 2 h, the reaction was quenched by addition of H_2O (10 ml), sat. $NaHCO_3$ soln. (30 ml) was added, and the mixture extracted with CH_2Cl_2 (3 × 20 ml). The extract was evaporated and submitted to short CC (CH_2Cl_2, then CH_2Cl_2/MeOH 95:5): 910 mg (90%) of **6d**. UV (MeOH): 259. ^{1}H-NMR ($CDCl_3$): 7.08 (H–C(4″)); 7.49 (H–C(5″)); 7.52 (H–C(3″)). ^{13}C-NMR ($CDCl_3$): 127.73, 128.81, 130.37, 137.52 (C(2″), C(3″), C(4″), C(5″)). MS: 618.2476 (M^+, $C_{27}H_{42}N_6O_5Si_2^+$, calc. 618.2474).

5. *2′-Deoxy-2′-(furan-2-carboxamido)-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6e**). To a soln. of **5** (800 mg, 1.57 mmol) in CH_2Cl_2/pyridine 3:1 (20 ml) at 0° was added dropwise furoyl chloride (0.185 ml, 245 mg, 1.88 mmol). After stirring for 1 h at r.t., H_2O (5 ml) was added, the mixture evaporated, and the crude residue purified by CC (CH_2Cl_2, then CH_2Cl_2/MeOH 95:5): 740 mg (78%) of **6e**. UV (MeOH): 257 (4.44). ^{1}H-NMR ($CDCl_3$): 6.50, 7.44, 7.45 (H–C(3″), H–C(4″), H–C(5″)). ^{13}C-NMR ($CDCl_3$): 112.17, 114.82 (C(3″), C(4″)); 144.30 (C(5″)); 147.32 (C(2″)). MS: 602.2704 (M^+, $C_{27}H_{42}N_6O_6Si_2^+$, calc. 602.2704).

6. *2′-(Benzenesulfonamido)-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6f**). To a cooled (0°) soln. of **5** (750 mg, 1.47 mmol) in CH_2Cl_2 (7.5 ml) and pyridine (2 ml) was added phenylsulfonyl chloride (0.26 ml, 360 mg, 2.03 mmol). The ice-bath was removed and stirring continued for 20 min at r.t. (TLC (CH_2Cl_2/MeOH 95:5): no **5** left). The mixture was evaporated and purified by CC (AcOEt): 759 mg (80%) of **6f**. UV (MeOH): 260 (4.16). ^{1}H-NMR ($CDCl_3$): 7.34 (2 H_m); 7.48 (H_p); 7.60 (2 H_o). ^{13}C-NMR ($CDCl_3$): 126.68 (2 C_o); 129.00 (2 C_m); 132.94 (C_p); 138.18 (C_{ipso}). MS: 648.2582 (M^+, $C_{28}H_{44}N_6O_6Si_2^+$, calc. 648.2606).

7. *2′-Deoxy-2′-(2-phenylacetamido)-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6g**). Phenylacetyl chloride (0.28 ml, 327 mg, 2.11 mmol) was added dropwise to a cold (0°) soln. of **5** (884 mg, 1.74 mmol) and 4-(dimethylamino)pyridine (638 mg, 5.22 mmol) in pyridine (15 ml). Stirring was continued for 1 h followed by evaporation and purification by CC (CH_2Cl_2, then CH_2Cl_2/MeOH 95:5): 795 mg (73%) of **6g**. UV (MeOH): 259. ^{1}H-NMR ($CDCl_3$): 3.58 (*A* of *AB*, $J(A,B) = -16.3$, 1 H, CH_2); 3.63 (*B* of *AB*, 1 H, CH_2); 7.20–7.35 (arom. H). ^{13}C-NMR ($CDCl_3$): 43.63 (CH_2CO); 127.47 (C_p); 129.05, 129.24 (2 C_o, 2 C_m); 134.09 (C_{ipso}). MS: 626.3068 (M^+, $C_{30}H_{46}N_6O_5Si_2^+$, calc. 626.3068).

8. *2′-Deoxy-2′-(3-methoxybenzamido)-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6h**). To a soln. of **5** (760 mg, 1.49 mmol) and 3-methoxybenzoic acid (304 mg, 2.0 mmol) in DMF (10 ml) at −20° were added *N*-hydroxysuccinimide (230 mg, 2 mmol) and dicyclohexylcarbodiimide (412 mg, 2.0 mmol). The mixture was stirred overnight at r.t., H_2O (5 ml) added, and the solvent evaporated. The residual oil was purified by CC (AcOEt): 584 mg (61%) of **6h**. Colorless foam. UV (MeOH): 258 (4.16). ^{1}H-NMR ($CDCl_3$): 3.85 (MeO); 7.28 (H–C(4″)); 7.28 (H–C(6″)); 7.35 (H–C(2″)); 7.35 (H–C(5″)). ^{13}C-NMR ($CDCl_3$): 55.32 (MeO); 111.99 (C(5″)); 118.43 (C(4″), C(6″)); 129.62 (C(2″)); 134.72 (C(1″)); 159.91 (C(3″)). MS: 642.3017 (M^+, $C_{30}H_{46}N_6O_6Si_2^+$, calc. 642.3077).

9. *2′-Deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-(thiophene-3-carboxamido)adenosine* (**6i**). To a suspension of **5** (860 mg, 1.69 mmol) and thiophene-3-carboxylic acid (260 mg, 2.03 mmol) in DMF (10 ml) at −20°, *N*-hydroxysuccinimide (230 mg, 2 mmol) and dicyclohexylcarbodiimide (412 mg, 2 mmol) were added. After 24 h stirring at r.t., the mixture was filtered, the solid material washed with AcOEt, and the combined filtrate evaporated. The residue was purified by CC (CH_2Cl_2/MeOH 95:5): 632 mg (60%) of **6i**. UV (MeOH): 253 (4.16). ^{1}H-NMR ($CDCl_3$): 7.34 (2 H of thiophene); 7.87 (1 H of thiophene). ^{13}C-NMR ($CDCl_3$): 126.50, 126.88, 128.50, 135.55 (C of thiophene). MS: 618.2476 (M^+, $C_{27}H_{42}N_6O_5Si_2^+$, calc. 618.2485).

10. *2′-Deoxy-2′-[(*RS*)-3-methylpentanamido]-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6j**). To a suspension of **5** (600 mg, 1.18 mmol) and (*RS*)-3-methylpentanoic and (0.25 ml, 233 mg, 2.0 mmol) in

DMF (10 ml) at −20° were added *N*-hydroxysuccinimide (230 mg, 2.0 mmol) and dicyclohexylcarbodiimide (412 mg, 2.0 mmol). The mixture was stirred for 30 min at 0° and overnight at r.t. After addition of H_2O (10 ml), the mixture was evaporated and purified by CC (CH_2Cl_2, then CH_2Cl_2/MeOH 95:5): 447 mg (62%) of **6j**. UV (MeOH): 259. ^{1}H-NMR ($CDCl_3$): 0.8–2.0 (complex *m*). ^{13}C-NMR ($CDCl_3$): 11.15 (C(5″)): 19.11 (*Me*C(3″)); 29.21 (C(4″)); 32.25 (C(3″)); 43.75 (C(2″)). MS: 606.3381 (M^+, $C_{28}H_{50}N_6O_5Si_2^+$, calc. 606.3398).

11. *2′-Acetamido-2′-deoxy-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**6k**). To a stirred soln. of **5** (1.80 g, 3.54 mmol) in CH_2Cl_2/pyridine 1:1 (30 ml), Ac_2O (1.0 ml) was added. After stirring for 2 h (TLC (CH_2Cl_2/MeOH 95:5): no **5** left), H_2O (5 ml) was added. Evaporation and short CC of the residual oil (CH_2Cl_2, then CH_2Cl_2/MeOH 95:5) yielded 1.66 g (85%) of **6k**. Colorless foam. UV (MeOH): 259 (4.18). ^{1}H-NMR ($CDCl_3$): 2.02 (Me). ^{13}C-NMR ($CDCl_3$): 22.92 (*Me*CO). MS: 550.2755 (M^+, $C_{24}H_{42}N_6O_5Si_2^+$, calc. 550.2779).

12. *2′-Benzamido-2′-deoxyadenosine* (**1a**). To a suspension of 2′-amino-2′-deoxydenosine (**4**, 200 mg, 0.751 mmol) in CH_2Cl_2 (5 ml) and pyridine (2 ml) at 0° was added dropwise benzoic anhydride (200 mg, 0.884 mmol) in CH_2Cl_2. After 1 h (TLC (CH_2Cl_2/MeOH 90:10): no **4** left), H_2O (5 ml) was added, the mixture concentrated, and after short CC, pure **1** (195 mg, 70%) isolated. White solid. M.p. 212–214° (dec.). UV (MeOH): 258 (4.20). ^{1}H-NMR ((D_6)DMSO): 7.43 (2 H_m); 7.51 (H_p); 7.82 (2 H_o). ^{13}C-NMR ((D_6)DMSO): 127.52, 128.23 (2 C_o, 2 C_m); 131.47 (C_p); 133.91 (C_{ipso}). Anal. calc. for $C_{17}H_{18}N_6O_4$ (370.4): C 55.13, H 4.90, N 22.69; found: C 54.60, H 4.98, N 22.31

13. *Preparation of* **1b–k.** 13.1. *General Procedure.* The protected nucleoside **6b–k** and NH_4F (10 equiv.) were dissolved in reagent-grade MeOH (20 ml) and stirred in an oil-bath at 60° for 3–6 h (TLC monitoring (CH_2Cl_2/MeOH 90:10)). TLC analysis clearly showed that the cleavage occurred in two steps. The soln. was evaporated, diluted with H_2O, and applied to a column (1.5 × 15 cm) of *Dowex 1X2* (OH^- form; H_2O/MeOH 80:20, then H_2O/MeOH 1:1)[1]). Evaporation gave compounds **1b–j** as crystalline material from EtOH (the more polar **1k** from MeOH).

13.2. *2′-Deoxy-2′-(4-methylbenzamido)adenosine* (**1b**). Yield 65% (based on **5**). M.p. 218–221° (dec.). UV (MeOH): 249 (4.32). ^{1}H-NMR ((D_6)DMSO): 2.32 (Me); 7.23 (2 H_m); 7.72 (2 H_o). ^{13}C-NMR ((D_6)DMSO): 21.05 (Me); 127.61 (2 C_o); 128.81 (2 C_m); 131.14 (C_{ipso}); 141.46 (C_p). Anal. calc. for $C_{18}H_{20}N_6O_4$ (384.4): C 56.24, H 5.24, N 21.86; found: C 55.94, H 5.24, N 21.52.

13.3. *2′-Deoxy-2′-(2-phenoxyacetamido)adenosine* (**1c**). Yield 55% (based on **5**). M.p. 199° (dec.). UV (MeOH): 260 (4.19). ^{1}H-NMR ((D_6)DMSO): 4.46 (CH_2); 6.82 (H_o); 6.92 (H_p); 7.21 (2 H_m); $J(2'',3'') \approx J(3'',4'') \approx J(4'',5'') \approx J(5'',6'') \approx 8.0$. ^{13}C-NMR (($D_6$)DMSO): 66.63 ($CH_2$CO); 114.66 ($C_o$); 121.24 ($C_p$); 129.48 ($C_m$); 157.61 ($C_{ipso}$). Anal. calc. for $C_{18}H_{20}N_6O_5 \cdot ½H_2O$ (409.4): C 52.81, H 5.17, N 20.53; found: C 53.23, H 5.14, N 20.56.

13.4. *2′-Deoxy-2′-(thiophene-2-carboxamido)adenosine* (**1d**). Yield 80% (based on **5**). M.p. 211.5–213° (dec.). UV (MeOH): 259 (4.39). ^{1}H-NMR ((D_6)DMSO): 7.12 (H−C(4″)); 7.73 (H−C(3″)); 7.85 (H−C(5″)); $J(3'',4'') = 3.7$, $J(3'',5'') \approx 1$, *H*, $(4'',5'') \approx 5$. ^{13}C-NMR ((D_6)DMSO): 127.89, 129.00, 131.28, 139.12 (C(2″), C(3″), C(4″), C(5″)). Anal. calc. for $C_{15}H_{16}N_6O_4S$ (376.4): C 47.87, H 4.28, N 22.33; found: C 47.98, H 4.39, N 22.39.

13.5. *2′-Deoxy-2′-(furan-2-carboxamido)adenosine* (**1e**). Yield 70% (based on **5**). M.p. 215.5–216° (dec.). UV (MeOH): 257 (4.42). ^{1}H-NMR ((D_6)DMSO): 6.60 (H−C(5″)); 7.10 (H−C(3″)); 7.83 (H−C(4″)); $J(3'',4'') = 3.6$, $J(4'',5'') = 1.8$. ^{13}C-NMR ((D_6)DMSO): 112.06, 114.32 (C(3″), C(4″)); 145.35 (C(5″)); 147.21 (C(2″)). Anal. calc. for $C_{15}H_{16}N_6O_5$ (360.3): C 50.00, H 4.48, N 23.32; found: C 49.38, H 4.55, N 22.98.

13.6. *2′-(Benzenesulfonamido)-2′-deoxyadenosine* (**1f**). Yield 73% (based on **5**). M.p. 219–221° (dec.). UV (MeOH): 261 (4.13). ^{1}H-NMR ((D_6)DMSO): 7.11 (2 H_m); 7.24 (H_p); 7.39 (2 H_o). ^{13}C-NMR ((D_6)DMSO): 125.36 (2 C_o); 128.17 (2 C_m); 131.56 (C_{ipso}); 140.83 (C_p). Anal. calc. for $C_{16}H_{18}N_6O_5S$ (406.4): C 47.28, H 4.46, N 20.68; found: C 47.04, H 4.47, N 20.46.

13.7. *2′-Deoxy-2′-(2-phenylacetamido)adenosine* (**1g**). Yield 65% (based on **5**). M.p. 201–202° (dec.). UV (MeOH): 259 (4.13). ^{1}H-NMR ((D_6)DMSO): 3.39 (*A* of *AB*, $J(A,B) = -11.0$, 1 H, CH_2); 3.45 (*B* of *AB*, 1 H, CH_2); 7.03, 7.14, 7.15 (Ph). ^{13}C-NMR ((D_6)DMSO): 41.84 (CH_2); 126.21 (C_p); 128.04, 128.83 (2 C_o, 2 C_m); 136.04 (C_{ipso}). Anal. calc. for $C_{18}H_{20}N_6O_4 \cdot ½H_2O$ (393.4): C 54.96, H 5.38, N 21.36; found: C 54.89, H 5.44, N 21.02.

13.8. *2′-Deoxy-2′-(3-methoxybenzamido)adenosine* (**1h**). Yield 51% (based on **5**). M.p. 206–208° (dec.). UV (MeOH): 258 (4.23). ^{1}H-NMR ((D_6)DMSO): 3.77 (MeO); 7.08 (H−C(4″)); 7.34 (H−C(5″)); 7.35 (H−C(2″)); 7.40 (H−C(6″)). ^{13}C-NMR ((D_6)DMSO): 55.35 (MeO); 112.89 (C(5″)); 117.23 (C(4″)); 119.75 (C(6″)); 129.39 (C(2″)); 135.38 (C(1″)); 159.15 (C(3″)). Anal. calc. for $C_{18}H_{20}N_6O_5$ (400.4): C 54.00, H 5.03, N 20.99; found: C 53.97, H 5.08, N 20.83.

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[1]) To decrease the retention time of the more acidic sulfonamido compound **1f**, it was eluted from the resin with H_2O/MeOH/AcOH 50:50:1.

13.9. *2′-Deoxy-2′-(thiophene-3-carboxamido)adenosine* (**1i**). Yield 50% (based on **5**). M.p. 206.5° (dec.). UV (MeOH): 253 (4.16). ^{1}H-NMR ((D_6)DMSO): 7.47 (H–C(5″)); 7.55 (H–C(4″)); 8.18 (H–C(2″)); $J(2'',4'') = 1$, $J(2'',5'') = 3.2$, $J(4'',5'') = 5.0$. ^{13}C-NMR ((D_6)DMSO): 126.66, 127.07, 129.41, 137.08 (C(2″), C(3″), C(4″), C(5″)). Anal. calc. for $C_{15}H_{16}N_6O_4S$ (376.4): C 47.87, H 4.28, N 22.33; found: C 47.85, H 4.31, N 22.10.

13.10. *2′-Deoxy-2′-[(RS)-3-methylpentanamido]adenosine* (**1j**). Yield 53% (based on **5**). M.p. 208° (dec.). UV (MeOH): 259 (4.17). ^{1}H-NMR ((D_6)DMSO): *Table 4*. ^{13}C-NMR ((D_6)DMSO): 10.95 (C(5″)); 18.90 (*Me*–C(3″)); 28.79 (C(4″)); 31.56 (C(3″)); 42.39 (C(2″)). Anal. calc. for $C_{16}H_{24}N_6O_4$ (364.4): C 52.74, H 6.64, N 23.06; found: C 52.93, H 6.71, N 22.42.

13.11. *2′-Acetamido-2′-deoxyadenosine* (**1k**). Yield 78% (based on **5**). M.p. 206.5–207.5° (dec.). UV (MeOH): 259 (4.18). ^{1}H-NMR ((D_6)DMSO): 1.77 (Me). ^{13}C-NMR ((D_6)DMSO): 22.56 (Me). Anal. calc. for $C_{12}H_{16}N_6O_4$ (308.3): C 46.75, H 5.23, N 27.26; found: C 46.70, H 5.27, N 27.07.

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