

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

by Serge Van Calenbergh, Elfride Van Den Eeckhout, and Piet Herdewijn*

Laboratory of Medicinal Chemistry (FFW), University of Ghent, Harelbekestraat 72, B-9000 Gent

and André De Bruyn

Laboratory of Organic Chemistry, University of Ghent, Krijgslaan 281, B-9000 Gent

and Christophe Verlinde and Wim Hol

Department of Biological Structure and Biomolecular Structure Program (S.M.), University of Washington, Seattle, WA 98195, USA

and Mia Callens

International Institute for Cellular and Molecular Pathology, Research Unit for Tropical Diseases, avenue Hippocrate 74, B-1200 Brussels

and Arthur Van Aerschot and Jef Rozenski

Laboratory of Medicinal Chemistry, Rega Institute, Catholic University of Leuven, Minderbroedersstraat 10, B-3000 Leuven

(20.X.93)

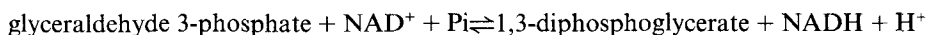
A series of 2'-benzamido-2'-deoxyadenosine analogues were synthesized in an effort to find new lead structures for the treatment of sleeping sickness. The 2'-deoxy-2'-(3-methoxybenzamido)adenosine (**1h**) was proved to be a selective inhibitor of the parasite glyceraldehyde 3-phosphate dehydrogenase 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 protozoan 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.

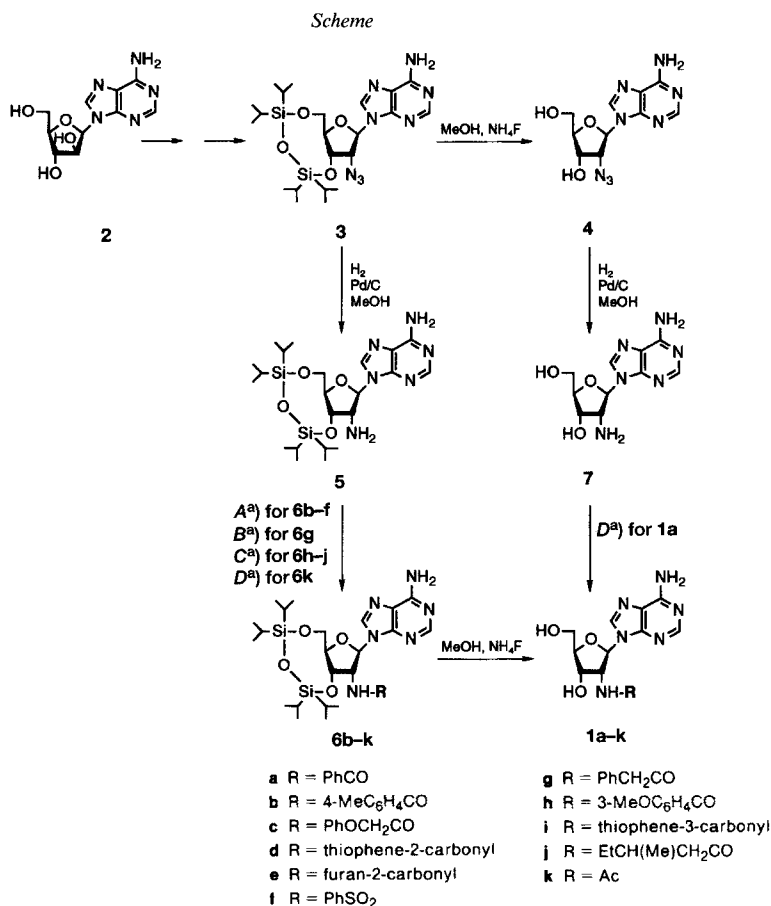
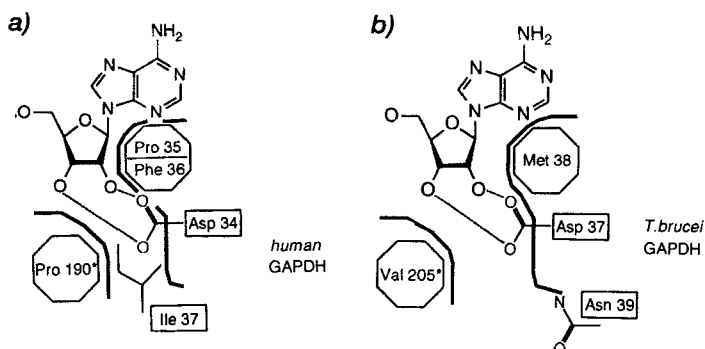
Trypanosomes exhibit several unusual features [4] which can be exploited for rational drug design. One of them is the fact that the bloodstream form of trypanosomes is completely dependent on glycolysis to the stage of pyruvate as the sole source of energy supply. These glycolytic reactions are sequestered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



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 (hGADPH) 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 hGADPH and gGADPH (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 gGADPH 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 gGADPH, 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 gGADPH to 105° in human GAPDH, ultimately leading to a different loop conformation. Therefore, only gGADPH 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



a) *Method A*: RCOCl, CH₂Cl₂, Py. *Method B*: RCOCl, Py, Me₂NC₅H₄N. *Method C*: RCOOH, DMF, DCC, *N*-hydroxysuccinimide. *Method D*: (RCO)₂O, CH₂Cl₂, 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-tetraisopropylsiloxane-1,3-diyl)- β -D-arabinofuranosyl]adenine. The N₃ group was introduced in the 2'-position using trifluoromethanesulfonyl chloride and NaN₃ ($\rightarrow$ **3** in 68% yield) [13]. Deprotection with NH₄F/MeOH yielded **4**. The N₃ function of **3** was reduced with H₂ in MeOH in the presence of Pd/C without loss of the O[(i-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 CH₂Cl₂ or

Table 1. ¹H-NMR Chemical Shifts [ppm] and Coupling Constants [Hz] for the O[(i-Pr)₂Si]₂-Protected Nucleoside Derivatives **5** and **6b–k** in CDCl₃

	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.

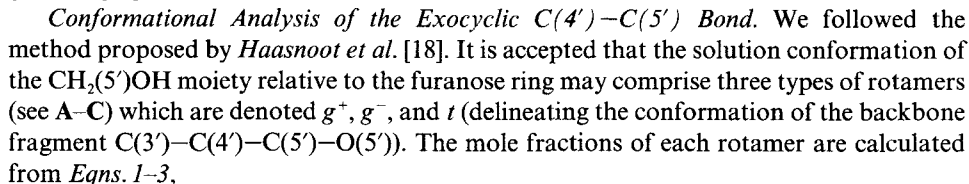


Table 2. ^1H -NMR Chemical Shifts [ppm] and Coupling Constants [Hz] for the Nucleoside Derivatives **1a–k** in (D_6)DMSO

	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
	$J(1',2')$	$J(2',3')$	$J(2',\text{NH})$	$J(3',4')$	$J(3',\text{OH–C}(3'))$	$J(4',5'A)$	$J(4',5'B)$	$J(5'A,\text{OH–C}(5'))$	$J(5'B,\text{OH–C}(5'))$	$J(5'A,5'B)$		
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-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 $\delta(\text{H}_A\text{--C}(5')) > \delta(\text{H}_B\text{--C}(5'))$ and $J(4',5'A) < J(4',5'B)$, the most shielded proton (H_B–C(5')) is H_{pro-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-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-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-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*).

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

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. $A\chi_i$ is the difference in *Huggins* electronegativity between the H-atom and the substituent and is given by *Eqn. 5*.

$$A\chi_i^{\text{subst}} = A\chi^\alpha - 0.19 \Sigma A\chi^\beta \quad (5)$$

For O, $A\chi$ 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 2E) [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 $^1J(\text{H},\text{H})$ 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 field in the 'Hyper Chem. Release 2' computer programme) with constraints on $\Phi_{\text{H,H}}$. One set is found: $P = 150^\circ$, $\Phi_m = 37.2^\circ$, 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 $P = 162^\circ$ and $\Phi_m = 35^\circ$, indeed a set of values very close to the set extracted by us, although these data are not always evident.

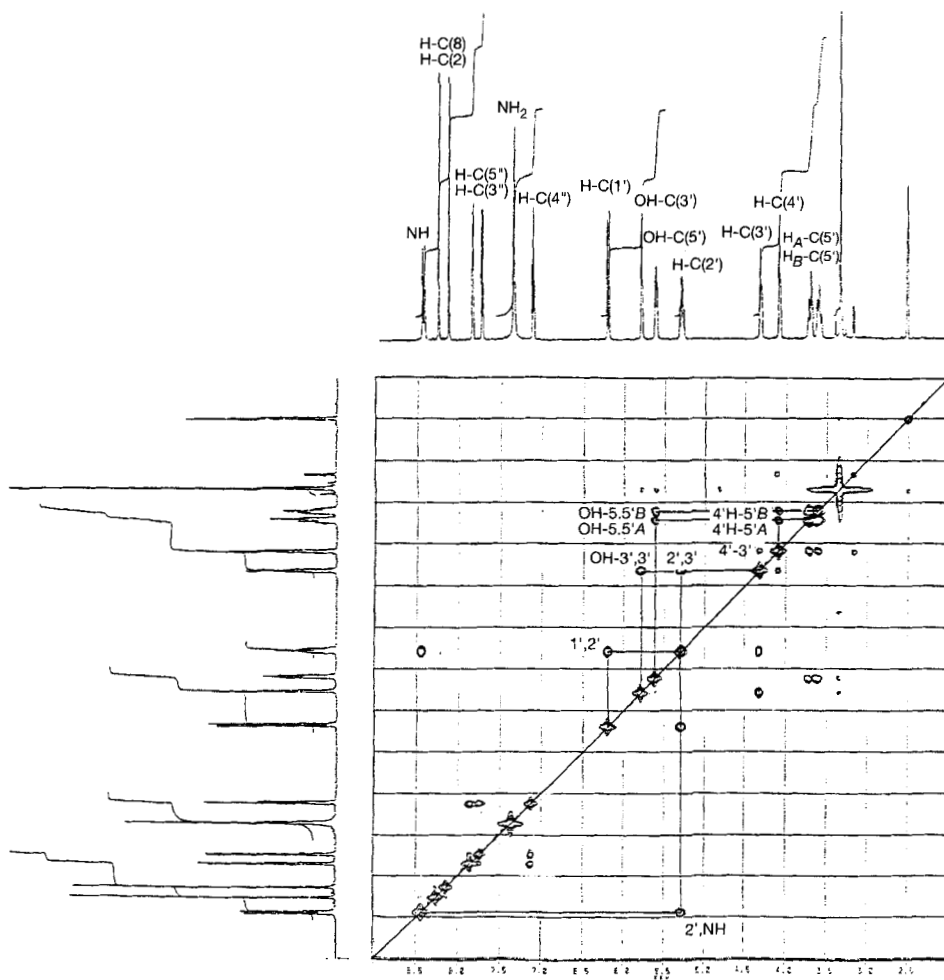


Fig. 2. $^1\text{H}, ^1\text{H}$ -COSY-45 Spectrum of compound **1d** in $(\text{D}_6)\text{DMSO}$. The connectivities between $\text{H}-\text{C}(1')$, $\text{H}-\text{C}(2')$, $\text{NH}-\text{C}(2')$, $\text{H}-\text{C}(3')$, $\text{OH}-\text{C}(3')$, and $\text{H}-\text{C}(4')$ and the cross-peaks of $\text{H}-\text{C}(4')$ and $\text{OH}-\text{C}(5')$ with $\text{H}_\text{A}-\text{C}(5')$ and $\text{H}_\text{B}-\text{C}(5')$ 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 $\cdots$ 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 (D₆)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-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].

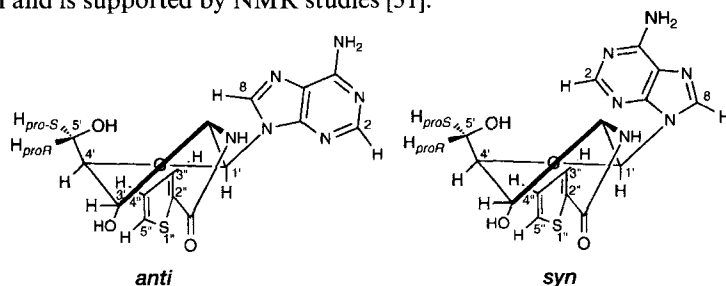


Fig. 3. 'anti'- and 'syn'-Conformation of the base relative to the sugar moiety in compound **1d**

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 ¹H-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 ¹³C-NMR Spectra. The resonances in the ¹H-noise-decoupled ¹³C-NMR spectra were first assigned by comparison with the data given in [33]. These assignments were verified by a ¹³C, ¹H heteronuclear correlated experiment, where the ¹³C-NMR resonances were assigned from the knowledge of the ¹H-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 ¹H-NMR resonance at the lowest frequency is linked to the C-atom which resonates at the highest frequency in the ¹³C-NMR spectrum. The other ¹³C-NMR resonances for adenine were assigned by comparison with known data [33]. All other ¹³C-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. ¹H-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)}
	<i>J</i> (2'',3'')	<i>J</i> (2'',3'')	<i>J</i> (2'',2'' _B)	<i>J</i> (3'',Me–C(3''))	<i>J</i> (4'',4'' _B)	<i>J</i> (4'',5'') ≈ <i>J</i> (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'')CH₂(4'')CH(3'')(Me)CH₂(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.

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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 (1 g/l). NMR Spectra: Bruker-WH-360 spectrometer; ^1H : 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 (^1H : (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 $90^\circ(^1\text{H})\text{-}t_1\text{-}90^\circ(^1\text{H})\text{-}t_2$ 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 ; $90^\circ(^1\text{H})$ pulse of 5.0 μs . ^{13}C , ^1H Heteronuclear correlated experiment: Bax-Morris sequence [35], i.e. $90^\circ(^1\text{H})\text{-}t_1/2\text{-}180^\circ(^{13}\text{C})\text{-}t_1/2\text{-}A_1\text{-}90^\circ(^1\text{H})\text{-}90^\circ(^{13}\text{C})\text{-}A_2\text{-}t_2$; $90^\circ(^1\text{H})$ pulse of 8.2 μs , $90^\circ(^{13}\text{C})$ pulse of 10.2 μs ; A_1 optimized to 3.2 ms and A_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. $90^\circ(^1\text{H})\text{-}t_1\text{-}90^\circ(^1\text{H})\text{-}\tau_m\text{-}A_1\text{-}90^\circ(^1\text{H})\text{-}t_2$; relaxation delay of 1.2 s, $90^\circ(^1\text{H})$ 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-tetraisopropylidisiloxane-1,3-diyl)adenosine (**5**). A soln. of 2'-azido-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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/m² 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^+ , $\text{C}_{22}\text{H}_{40}\text{N}_6\text{O}_4\text{Si}_2^+$, calc. 508.2647).

2. *2'-Deoxy-2'-(4-methylbenzamido)-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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 CH₂Cl₂ (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). ¹H-NMR (CDCl₃): 2.40 (Me); 7.25 (2 H_m); 7.67 (2 H_o). ¹³C-NMR (CDCl₃): 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⁺, C₃₀H₄₆N₆O₅Si₂⁺, calc. 626.3112).

3. *2'-Deoxy-2'-(2-phenoxyacetamido)-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)adenosine (6c)*. A soln. of **5** (712 mg, 1.40 mmol) in CH₂Cl₂ (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 (CH₂Cl₂/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). ¹H-NMR (CDCl₃): 4.47 A of AB (*J*(A,B) = −15.1, 1 H, CH₂); 4.56 (B of AB, 1 H, CH₂); 6.88 (2 H_o); 7.02 (H_p); 7.30 (2 H_m). ¹³C-NMR (CDCl₃): 67.21 (CH₂CO); 114.38 (2 C_o); 122.12 (C_p); 129.70 (2 C_m); 156.97 (C_{ipso}). MS: 642.3017 (M⁺, C₃₀H₄₆N₆O₆Si₂⁺, calc. 642.3066).

4. *2'-Deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-2'-(thiophene-2-carboxamido)adenosine (6d)*. To a cooled (ice-bath) soln. of **5** (830 mg, 1.63 mmol) in CH₂Cl₂ (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 H₂O (10 ml), sat. NaHCO₃ soln. (30 ml) was added, and the mixture extracted with CH₂Cl₂ (3 × 20 ml). The extract was evaporated and submitted to short CC (CH₂Cl₂, then CH₂Cl₂/MeOH 95:5): 910 mg (90%) of **6d**. UV (MeOH): 259. ¹H-NMR (CDCl₃): 7.08 (H-C(4'')); 7.49 (H-C(5'')); 7.52 (H-C(3'')). ¹³C-NMR (CDCl₃): 127.73, 128.81, 130.37, 137.52 (C(2''), C(3''), C(4''), C(5'')). MS: 618.2476 (M⁺, C₂₇H₄₂N₆O₅Si₂⁺, calc. 618.2474).

5. *2'-Deoxy-2'-(furan-2-carboxamido)-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)adenosine (6e)*. To a soln. of **5** (800 mg, 1.57 mmol) in CH₂Cl₂/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., H₂O (5 ml) was added, the mixture evaporated, and the crude residue purified by CC (CH₂Cl₂, then CH₂Cl₂/MeOH 95:5): 740 mg (78%) of **6e**. UV (MeOH): 257 (4.44). ¹H-NMR (CDCl₃): 6.50, 7.44, 7.45 (H-C(3''), H-C(4''), H-C(5'')). ¹³C-NMR (CDCl₃): 112.17, 114.82 (C(3''), C(4'')); 144.30 (C(5'')); 147.32 (C(2'')). MS: 602.2704 (M⁺, C₂₇H₄₂N₆O₆Si₂⁺, calc. 602.2704).

6. *2'-(Benzenesulfonamido)-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)adenosine (6f)*. To a cooled (0°) soln. of **5** (750 mg, 1.47 mmol) in CH₂Cl₂ (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 (CH₂Cl₂/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). ¹H-NMR (CDCl₃): 7.34 (2 H_m); 7.48 (H_p); 7.60 (2 H_o). ¹³C-NMR (CDCl₃): 126.68 (2 C_o); 129.00 (2 C_m); 132.94 (C_p); 138.18 (C_{ipso}). MS: 648.2582 (M⁺, C₂₈H₄₄N₆O₆Si₂⁺, calc. 648.2606).

7. *2'-Deoxy-2'-(2-phenylacetamido)-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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 (CH₂Cl₂, then CH₂Cl₂/MeOH 95:5): 795 mg (73%) of **6g**. UV (MeOH): 259. ¹H-NMR (CDCl₃): 3.58 (A of AB, *J*(A,B) = −16.3, 1 H, CH₂); 3.63 (B of AB, 1 H, CH₂); 7.20–7.35 (arom. H). ¹³C-NMR (CDCl₃): 43.63 (CH₂CO); 127.47 (C_p); 129.05, 129.24 (2 C_o, 2 C_m); 134.09 (C_{ipso}). MS: 626.3068 (M⁺, C₃₀H₄₆N₆O₅Si₂⁺, calc. 626.3068).

8. *2'-Deoxy-2'-(3-methoxybenzamido)-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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., H₂O (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). ¹H-NMR (CDCl₃): 3.85 (MeO); 7.28 (H-C(4'')); 7.28 (H-C(6'')); 7.35 (H-C(2'')); 7.35 (H-C(5'')). ¹³C-NMR (CDCl₃): 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⁺, C₃₀H₄₆N₆O₆Si₂⁺, calc. 642.3077).

9. *2'-Deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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 (CH₂Cl₂/MeOH 95:5): 632 mg (60%) of **6i**. UV (MeOH): 253 (4.16). ¹H-NMR (CDCl₃): 7.34 (2 H of thiophene); 7.87 (1 H of thiophene). ¹³C-NMR (CDCl₃): 126.50, 126.88, 128.50, 135.55 (C of thiophene). MS: 618.2476 (M⁺, C₂₇H₄₂N₆O₅Si₂⁺, calc. 618.2485).

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

1) To decrease the retention time of the more acidic sulfonamido compound **1f**, it was eluted from the resin with H₂O/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). ¹H-NMR ((D₆)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. ¹³C-NMR ((D₆)DMSO): 126.66, 127.07, 129.41, 137.08 (C(2''), C(3''), C(4''), C(5'')). Anal. calc. for C₁₅H₁₆N₆O₄S (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). ¹H-NMR ((D₆)DMSO): Table 4. ¹³C-NMR ((D₆)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 C₁₆H₂₄N₆O₄ (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). ¹H-NMR ((D₆)DMSO): 1.77 (Me). ¹³C-NMR ((D₆)DMSO): 22.56 (Me). Anal. calc. for C₁₂H₁₆N₆O₄ (308.3): C 46.75, H 5.23, N 27.26; found: C 46.70, H 5.27, N 27.07.

REFERENCES

- [1] WHO, 'TDR Seventh Program Report, Tropical Disease Research', WHO, Geneva, 1985.
- [2] W. E. Gutteridge, *Br. Med. Bull.* **1985**, *41*, 162.
- [3] D. H. Molyneux, R. W. Ashford, 'The Biology of Trypanosoma and Leishmania', Taylor and Francis, London, 1983.
- [4] F. R. Opperdoes, *Br. Med. Bull.* **1985**, *41*, 130.
- [5] F. R. Opperdoes, *Ann. Rev. Microbiol.* **1987**, *41*, 127.
- [6] P. A. M. Michels, A. Poliszczak, K. A. Osinga, O. Misset, J. Van Beeumen, R. K. Wierenga, P. Borst, F. R. Opperdoes, *EMBO J.* **1986**, *5*, 1049.
- [7] P. A. M. Michels, M. Marchand, L. Kohl, S. Allert, R. K. Wierenga, F. R. Opperdoes, *Eur. J. Biochem.* **1991**, *198*, 421.
- [8] F. M. D. Vellieux, J. Hajdu, C. L. M. J. Verlinde, H. Groendijk, R. J. Read, T. J. Greenhough, J. W. Campbell, K. H. Kalk, J. A. Littlechild, H. C. Watson, W. G. J. Hol, *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 2355.
- [9] W. D. Mercer, S. I. Winn, H. C. Watson, *J. Mol. Biol.* **1976**, *104*, 277.
- [10] R. J. Read, unpublished work (Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, ABT6G2H7, Canada).
- [11] C. L. M. J. Verlinde, M. Callens, S. Van Calenbergh, A. Van Aerschot, P. Herdewijn, V. Hannaert, P. A. M. Michels, F. R. Opperdoes, W. Hol, submitted to *J. Med. Chem.*
- [12] W. T. Markiewicz, *J. Chem. Res. (M)* **1979**, 181.
- [13] M. J. Robins, S. D. Hawrelak, A. E. Hernández, S. F. Wnuk, *Nucleos. Nucleot.* **1992**, *11*, 821.
- [14] J. Wengel, M. S. Motawia, E. B. Pedersen, C. M. Nielsen, *J. Heterocycl. Chem.* **1992**, *29*, 5.
- [15] W. Zhang, M. J. Robins, *Tetrahedron Lett.* **1992**, *33*, 1177.
- [16] P. Kumar, L. I. Wiebe, *Nucleos. Nucleot.* **1992**, *11*, 401.
- [17] L. M. Jackman, S. Sternhell, 'Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry', 2 edn., Pergamon Press, Oxford, 1969, p. 306.
- [18] C. A. G. Haasnoot, F. A. A. M. de Leeuw, H. P. M. de Leeuw, C. Altona, *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 576.
- [19] M. Remin, D. Shugar, *Biochem. Biophys. Res. Commun.* **1972**, *48*, 636.
- [20] W. Saenger, 'Principles of Nucleic Acid Structure', Springer Verlag, New York, 1983, p. 79.
- [21] F. J. M. Van de Ven, C. W. Hilbers, *Eur. J. Biochem.* **1988**, *178*, 1.
- [22] C. Altona, *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413.
- [23] L. D. Hall, P. R. Steiner, C. Pedersen, *Can. J. Chem.* **1970**, *48*, 1155.
- [24] C. Altona, M. Sundaralingam, *J. Am. Chem. Soc.* **1972**, *94*, 8205.
- [25] F. A. A. M. de Leeuw, C. Altona, *J. Chem. Soc., Perkin Trans. 2* **1982**, 375.
- [26] S. Uesugi, H. Miki, M. Ikehara, H. Iwahashi, Y. Kyogoku, *Tetrahedron Lett.* **1979**, *42*, 4073.
- [27] C. A. G. Haasnoot, F. A. A. M. de Leeuw, H. P. M. de Leeuw, C. Altona, *Org. Magn. Reson.* **1981**, *15*, 43.
- [28] W. Guschlbauer, K. Jankowski, *Nucleic Acids Res.* **1980**, *8*, 1421.
- [29] D. C. Rohrer, M. Sundaralingam, *J. Am. Chem. Soc.* **1970**, *92*, 4956.
- [30] H. P. M. de Leeuw, C. A. G. Haasnoot, C. Altona, *Isr. J. Chem.* **1980**, *20*, 108.
- [31] W. Saenger, 'Principles of Nucleic Acid Structure', Springer Verlag, New York, 1983, p. 71.
- [32] F. Lambein, J. K. Khan, C. Bécu, A. De Bruyn, *Phytochemistry* **1992**, *31*, 887.
- [33] H. Kalinowski, S. Berger, S. Braun, '¹³C-NMR-Spektroskopie', Georg Thieme Verlag, Stuttgart–New York, 1984, p. 399.
- [34] A. Bax, 'Two-Dimensional Nuclear Magnetic Resonance in Liquids', Reidel, Dordrecht, 1982, p. 78.
- [35] A. Bax, G. A. Morris, *J. Magn. Reson.* **1981**, *42*, 501.
- [36] S. Macura, K. Wüthrich, R. R. Ernst, *J. Magn. Reson.* **1982**, *46*, 269.