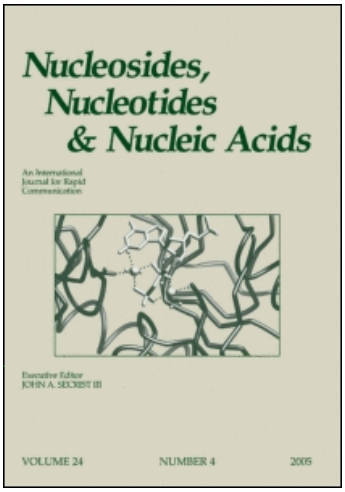

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1-Deaza-3'-O-methyladenosine: A Nucleoside with the *Syn*-conformation in the Solid State and in Solution

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1-DEAZA-3'-O-METHYLADENOSINE: A NUCLEOSIDE WITH THE *SYN*-CONFORMATION IN THE SOLID STATE AND IN SOLUTION

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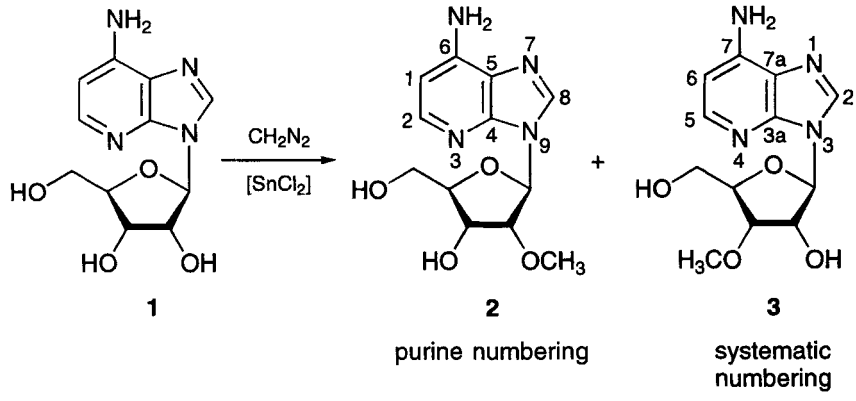
ABSTRACT: The 2'-*O*-methyl (**2**) and the 3'-*O*-methyl (**3**) derivatives of 1-deazaadenosine (**1**) were prepared. Single crystal X-ray analysis as well as ^{1}H and ^{13}C NMR studies were performed on the 3'-*O*-methyl-1-deazaadenosine **3**. In the solid state, the glycosyl torsion angle ($\chi = 64.7°$) is in the *syn*-range which is caused by an intramolecular (5')CH_2OH···N(3) hydrogen bond. The ribofuranose moiety adopts a ^{2}E (C-3'-*exo*; S) conformation and the orientation of the exocyclic C(4')-C(5') bond is +*sc* ($\gamma^{(+)g}$). The conformation in solution was found to be very similar to that in solid state. Whereas the 2'-*O*-methyl derivative of **1** is a strong inhibitor of adenosine deaminase the 3'-*O*-methyl derivative is neither inhibitor nor substrate.

INTRODUCTION

1-Deazaadenosine (**1**, purine numbering is used in the discussion) is a structural analogue of adenosine which was first prepared by Jain[1] and the synthesis has been improved by Mizuno.[2] It is a strong inhibitor of adenosine deaminase[3] and has been incorporated into oligonucleotides by enzymatic and chemical methods.[4-7] The resulting oligonucleotides show unusual structures with a preference for Hoogsteen base-pairing[4,6-8] and were used as a structural probe for the hammerhead ribozyme.[9] On the basis of ^{13}C NMR and ^{1}H-^{1}H NOE difference spectra a high population of *syn*-conformers was suggested for 1-

deazaadenosine (**1**) implicating a hydrogen bond between the 5'-hydroxyl group and nitrogen-3.[5] This can be considered to be the result of the increased π-electron density of the pyridine system compared to the pyrimidine moiety of purine nucleosides.[5,10] Furthermore, it was shown that the N-glycosyl bond conformation of 1-deazapurine nucleosides strongly depends on the nature of the 6-substituents and a linear correlation of the *syn*-conformer population *vs.* the σ_{para} Hammett constants for the 6-substituents was established.[5]

In most of the cases the *syn*-conformation of a nucleoside is induced by an unfavourable steric and/or electronic interaction with the pentofuranose moiety which destabilises the *anti*-conformation.[11] For instance, the *syn*-conformation has been found in the solid state of 8-bromoadenosine,[12] 8-bromoguanosine[12] and 8-(α-hydroxy-isopropyl)adenosine[13] that was stabilized by the formation of the 5'-OH···N(3) hydrogen bond. Such a hydrogen bond was also detected in the crystal structure of inosine[14] pointing to the possible role of this interaction as a sole factor forcing the *syn*-conformation. However, the favourable proximity of the base N(3) atom and the 5'-hydroxyl group does not inevitably lead to the formation of a hydrogen bond (*cf.*, *e.g.*, the data[15-17]).



1 **2** **3**

purine numbering systematic numbering

During the course of our work on oligonucleotides containing 1-deazaadenosine[5-7] the 2'- and 3'-*O*-methyl derivatives **2** and **3** were prepared. A comparative NMR study of 1-deazaadenosine and its *O*-methyl derivatives revealed a close similarity of the conformational behaviour of these molecules. As compound **3** crystallises from acetonitrile

to afford crystals which were suitable for X-ray analysis, this compound was chosen to investigate the conformational properties in the solid state. The X-ray analysis of compound **3** provides direct evidence for a hydrogen bond between the 5'-hydroxyl group and nitrogen-3 forcing the base into *syn*-conformation.

RESULTS AND DISCUSSION

The 2'- and 3'-*O*-methyl-1-deazaadenosines **2** and **3** were prepared from 1-deazaadenosine (**1**) by treatment of compound **1** with diazomethane in the presence of $SnCl_2$/methanol according to a protocol of Robins *et al.*[18] Separation by ion-exchange column chromatography on Dowex 1-X2 (OH^-)[19] (see Scheme) afforded the isomers **2** and **3** in 20 and 72% yield. The structure of both isomers was proven by ^1H and ^{13}C NMR, and UV spectroscopy. It is noteworthy that the 5'-hydroxyl group of both isomers as well as the parent nucleoside display a doublet of doublets in their ^1H NMR spectra in d_6-DMSO solutions implying a constrained conformation about the C(4')-C(5') bond (Experimental Part). The latter points to the absence of free rotation on the NMR time scale about the C(5')-O(5') bond probably due to the involvement of this OH group in an intramolecular hydrogen bond. The ^{13}C NMR data (Tables 3 and 4) gave further confirmation of the structures of **2** and **3**. The proton-coupled ^{13}C NMR spectrum of isomer **2** shows splitting of the 2'-carbon signal owing to interaction with protons of the methyl group [$^3J(^{13}C,H)$ coupling]. In the case of isomer **3** the 3'-carbon signal is markedly broadened compared to the 2'-signal. Methylation of 3'-OH group resulted in deshielding of the α-carbon by 9.4 ppm compared to the parent nucleoside. Alternative methylation gave rise to the same downfield shift of the corresponding sugar carbon by 9.0 ppm (Table 3).

The assignments of the ^{13}C resonances of the base of compounds **1-3** are straightforward[20,21] (Table 3). The resonances of C(1) and C(2) display characteristic $^2J(^{13}C,H)$ spin couplings to their corresponding neighbour protons. Moreover, the C(1) resonances, such as C(5), display additional splittings into triplets owing to the interaction with the protons of the NH_2 group. The resonances of C(4) and C(8) show ^3J couplings ($^{13}C,H$) with H(1') besides the characteristic coupling patterns with H(2) and H(8) of the former and H(8) of the latter one.

Conformation in solid state

The structure of 3'-*O*-methy-1-deazaadenosine (**3**), as observed in the crystal structure by single-crystal X-ray diffraction, is shown in Figs. 1 and 2. Bond lengths and angles for com-pound **3** are listed in Table 1 and torsion angles in Table 2. The crystal parameters are summarised in the Experimental Section.

In the solid state compound **3** shows the *syn*-conformation. The glycosylic torsion angle (χ) is 64.7°. This conformation is stabilized by an intramolecular hydrogen bond between the 5'-hydroxyl group and the N(3) atom of the base [d O(5')···N(3) = 281.0 pm, angle O(5')-H···N(3) = 170.9°]. In addition to this intramolecular hydrogen bond, two intermolecular hydrogen bonds are formed to stabilise the crystal structure of 3'-*O*-methyl-1-deazaadenosine (**3**). The 2'-hydroxyl group acts as donor and acceptor in these hydrogen bonds.

The 1-deazapurine base of compound **3** is nearly planar. The deviations of its carbon and nitrogen atoms from the least-squares planes are in the range of +1.60(2) and -2.00(2) pm [C(1) = -1.50(2) pm; C(2) = 1.20(2) pm; N(3) = 1.90(2) pm; C(4) = 0.10(2) pm; C(5) = 1.90(2) pm; C(6) = -2.50(2) pm; N(7) = -2.80(2) pm; C(8) = -0.50(2) pm; N(9) = 3.40(2) pm]. The atoms N(6) and C(1') on the base are displaced from the plane of the carbon nitrogen skeleton by -7.50(4) pm and -23.00(3) pm.

Conformation in solution

The conformation of compound **3** in d_6-DMSO solution was studied by ^1H and ^{13}C NMR spectroscopy. Accurate determinations of the ^3J(H(4'),H(5')) and ^3J(H(4'),H(5")) coupling constants were, however, impossible owing to the presence of second-order effects and unresolved long-range couplings (*cf.*, *e.g.*, the data[22]). On the contrary, in D_2O solution these couplings can be readily measured and used for analysis of the C(4')-C(5') conformation. In order to compare the conformation of sugar moieties, the ^1H NMR spectra of compounds **1-3** were measured in D_2O solution. The conformational equilibrium of the furanose ring of isomer **3** in D_2O and d_6-DMSO solutions was found to be very similar (*vide infra*). An analogous conformational behaviour of a furanose ring independent on the solvent nature was previously documented.[22-25]

Stereoelectronic effects on the conformation of the pentofuranose moieties of nucleosides have intensively been studied during the last decade.[26-28] Conformations of

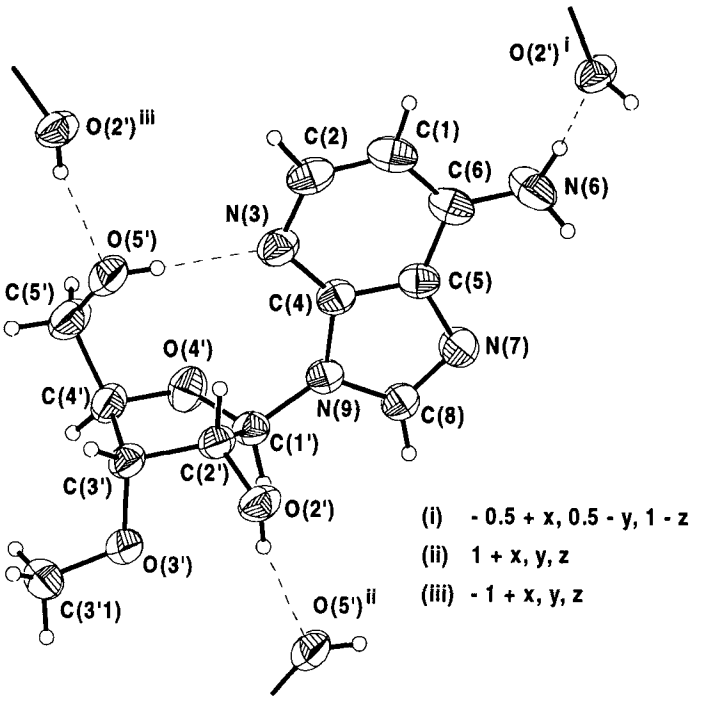


FIG. 1 Molecular structure of 3'-*O*-methyl-1-deazaadenosine **3** in the solid state. Anisotropic displacement ellipsoids representing the 50% probability density of the corresponding atoms are shown; hydrogens are drawn as spheres with arbitrary radius. Hydrogen bonding pattern within the solid-state structure of 3'-*O*-methyl-1-deazaadenosine (**3**); bond lengths: O(5')···N(3) = 281.0 pm; N(6)···O(2') = 293.1 pm; O(2')···O(5') = 273.7 pm; bond angles: O(5')-H(5')···N(3) = 170.9°; N(6)-H(6)···O(2') = 159.5°; O(2')-H(2')···O(5') = 158.6°.

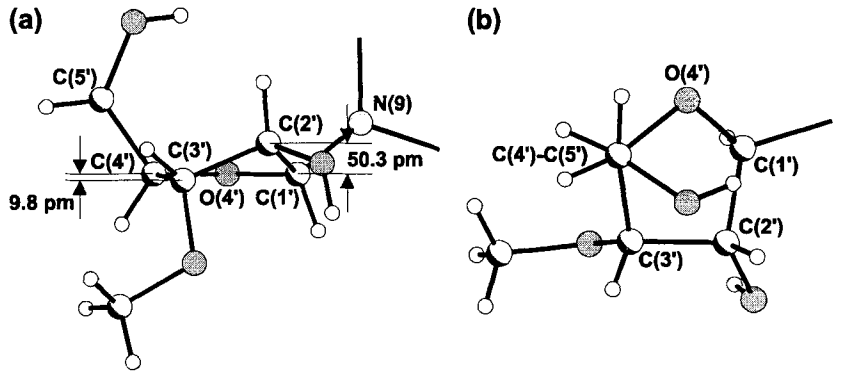


FIG. 2 (a) The sugar viewed end on parallel to the three-atom plane C(1')-O(4')-C(4'); thus the conformation of the sugar is C(2')-*endo*-C(3')-*exo*. (b) A projection down the C(5')-C(4') bond showing the γ^+ conformation [O(5')-C(5')-C(4')-C(3') = 47.5°]

TABLE 1 Bond lengths (pm) and angles (°) of 3'-*O*-methyl-1-deazaadenosine **3** [a].

C(1)-C(2)	137.9(4)	N(9)-C(1')	145.8(3)
C(1)-C(6)	138.2(4)	C(1')-O(4')	141.3(3)
C(2)-N(3)	134.0(3)	C(1')-C(2')	153.2(3)
N(3)-C(4)	133.7(3)	O(2')-C(2')	141.0(3)
C(4)-N(9)	137.9(3)	C(2')-C(3')	152.1(3)
C(4)-C(5)	138.3(3)	C(3')-O(3')	142.0(3)
C(5)-N(7)	138.7(3)	C(3')-C(4')	152.8(4)
C(5)-C(6)	140.7(3)	O(3')-C(31')	142.0(3)
C(6)-N(6)	135.4(3)	C(4')-O(4')	145.1(3)
N(7)-C(8)	131.0(3)	C(4')-C(5')	151.2(4)
C(8)-N(9)	136.7(3)	C(5')-O(5')	141.6(3)
C(2)-C(1)-C(6)	121.9(2)	C(4)-N(9)-C(1')	128.1(2)
N(3)-C(2)-C(1)	125.6(2)	O(6')-C(1')-N(9)	109.2(2)
C(4)-N(3)-C(2)	111.7(2)	O(6')-C(1')-C(2')	105.9(2)
N(3)-C(4)-N(9)	127.3(2)	N(9)-C(1')-C(2')	114.0(2)
N(3)-C(4)-C(5)	127.8(2)	O(2')-C(2')-C(3')	116.3(2)
N(9)-C(4)-C(5)	105.0(2)	O(2')-C(2')-C(1')	113.6(2)
C(4)-C(5)-N(7)	111.3(2)	C(3')-C(2')-C(1')	101.2(2)
C(4)-C(5)-C(6)	118.9(2)	O(3')-C(3')-C(2')	105.7(2)
N(7)-C(5)-C(6)	129.7(2)	O(3')-C(3')-C(4')	112.2(2)
N(6)-C(6)-C(1)	124.2(2)	C(2')-C(3')-C(4')	102.6(2)
N(6)-C(6)-C(5)	121.8(2)	C(31')-O(3')-C(3')	114.5(2)
C(1)-C(6)-C(5)	114.0(2)	O(4')-C(4')-C(5')	109.1(2)
C(8)-N(7)-C(5)	103.5(2)	O(4')-C(4')-C(3')	106.0(2)
N(7)-C(8)-N(9)	114.0(2)	C(5')-C(4')-C(3')	116.7(2)
C(8)-N(9)-C(4)	106.2(2)	O(5')-C(5')-C(4')	114.2(2)
C(8)-N(9)-C(1')	125.3(2)	C(1')-O(4')-C(4')	109.9(2)

[a] Purine numbering.

TABLE 2 Torsion angles (°) of the sugar moiety of 3'-*O*-methyl-1-deazaadenosine **3** [a].

C(4')-O(4')-C(1')-C(2')	ν_0	-20.0(2)
O(4')-C(1')-C(2')-C(3')	ν_1	35.3(2)
C(1')-C(2')-C(3')-C(4')	ν_2	-36.3(2)
C(2')-C(3')-C(4')-O(4')	ν_3	25.9(2)
C(3')-C(4')-O(4')-C(1')	ν_4	-3.8(2)
O(5')-C(5')-C(4')-C(3')	γ	47.4(4)
O(5')-C(5')-C(4')-O(4')		-72.7(3)
C(5')-C(4')-C(3')-O(3')	δ	151.3(2)
O(4')-C(1')-N(9)-C(4)	χ	64.7(3)
O(4')-C(1')-N(9)-C(8)		-123.7(2)

[a] Purine numbering.

TABLE 3 ^{13}C NMR Chemical shifts δ (ppm) of the 1-deazaadenosine derivatives **1-3** [a].

[b]	C(6)	C(5)	C(3a)	C(7a)	C(7)	C(2)
[c]	C(1)	C(2)	C(4)	C(5)	C(6)	C(8)
1[5]	102.4	144.2	146.5	123.8	147.4	140.0
2	102.4	144.3	146.4	123.6	147.3	139.7
3	102.3	144.1	146.4	123.8	147.4	139.9
	C(1')	C(2')	C(3')	C(4')	C(5')	OMe
1[5]	88.7	72.9	71.1	86.2	62.1	-
2	86.4	81.9	69.0	86.7	61.8	57.3
3	88.6	72.4	80.5	83.6	62.1	57.7

[a] Temp., 303 K; solvent, d_6-DMSO; 125 MHz. [b] Systematic numbering. [c] Purine numbering.

TABLE 4 C,H-Coupling constants (Hz) of the 1-deazaadenosine derivatives **1-3** [a].

	1	**2**	**3**
^1J(C(1),H(1))	160.7	160.7	160.3
^2J(C(1),H(2))	14.1	14.0	13.9
^3J(C(1),NH_2)	4.2	5.8	4.8
^1J(C(2),H(2))	174.6	174.9	174.9
^2J(C(2),H(1))	2.1	2.0	2.8
^3J(C(4),H(2))	14.7	14.0	14.2
^3J(C(4),H(8))	4.6	3.9	4.3
^3J(C(4),H(1'))	4.6	3.9	4.3
^3J(C(5),H(1))	11.3	11.1	10.9
^3J(C(5),NH_2)	5.4	5.5	5.6
^3J(C(5),H(8))	5.4	5.5	5.6
^2J(C(6),H(1))	8.3	8.3	8.3
^1J(C(8),H(8))	211.0	211.2	211.1
^3J(C(8),H(1'))	2.8	3.3	3.5
^1J(C(1'),H(1'))	162.9	163.6	164.9
^1J(C(2'),H(2'))	147.1	146.5	146.9
^1J(C(3'),H(3'))	148.8	150.8	150.1
^1J(C(4'),H(4'))	150.0	148.8	147.4
^1J(C(5'),H(5'))	128.0	140.6	140.8
^1J(C(Me),H(Me))	-	142.0	141.4
^3J(C(Me),H(α))	-	3.4	4.4

[a] Temp., 303 K; solvent, d_6-DMSO; 125 MHz.

the ribofuranose moieties of compounds **1-3** were investigated by using the PSEUROT analysis of vicinal proton-proton coupling constants in furanose rings (version 6.2; C.A. Altona, Gorlaeus Laboratories, Leiden, The Netherlands). The pseudorotational parameters $P_s = 167.1°$ and $\phi_m = 37.2°$ were calculated on the basis of the solid-state structure data of compound **3** using equations of the PSEUROT manual (p. 10; eqs. 11 and 12). Furthermore, we have measured the ^{1}H NMR spectra of the aforementioned

compounds and the corresponding vicinal ^3J(H,H) coupling constants were analysed with the WinDaisy program package (Bruker) (Table 5). Consideration of the ^3J(H,H) couplings of the pentofuranose moiety shows that the S-conformation (^2E; C-3'-*exo*) is the most populated one. Therefore, the aforementioned pseudorotational parameters were used for the predominant S-conformer, whereas the parameters for the minor N-conformer were taken from the PSEUROT manual and fixed during calculations. The populations of the staggered rotamers across the C(4')-C(5') bond (torsion angle γ) were calculated from ^3J(H(4'),H(5')) and ^3J(H(4'),H(5")) according to Haasnot *et al.*[29] The data are presented in Table 6.

It should be stressed that *O*-methyl derivatives **2** and **3** as well as the parent nucleoside **1** show close conformational similarity. The most striking is the correlation between the predominant S-conformation of the furanose ring (81-82%) and the γ^+ orientation about the C(4')-C(5') exocyclic bond (76.5-79%) (Table 6).

The conformation of the base about the glycosyl bond was investigated by means of ^{13}C NMR and ^{1}H-^{1}H NOE difference spectroscopy. In the former case, the vicinal ^3J(C(4),H(1')) and ^3J(C(8),H(1')) couplings unambiguously proves the predominant *syn*-conformation about the glycosyl bond (Table 4).[20,21] These data are in reasonable agreement with nuclear Overhauser enhancements for both the H(1') and H(2') resonances upon irradiation of H(8) (Table 7).[5] Besides some secondary nuclear Overhauser enhancements, the following NOEs were detected upon irradiation of the H(2') proton: 2.8% [H(8)], 2.9% (5'-OH), 5.0% (2'-OH); no NOE was observed at H(2). Again, these conformational peculiarities correspond well with the structure of compound **3** in the solid state. From X-ray data it follows that the distances between H(8) of the base and H(1') and H(2') of the sugar moiety are 247 and 428 pm, respectively. The population of the *syn*-conformation was calculated according to the previously published[30] equation. As it can be seen from Table 7, both *O*-methyl isomers exhibit the *syn*-conformation similar to that observed in the solid state.

On the basis of these data one may hypothesize that the (5')$CH_2OH\cdots$N(3) hydrogen bond is a crucial factor influencing the spatial arrangement of compounds **1-3**. The formation of this hydrogen bond is supported by the *trans* and *gauche* ^3J(5'OH,H5') and ^3J(5'OH,H5") coupling constants in d_6-DMSO solution (see Experimental). The question

TABLE 5 ^{1}H NMR Chemical shifts (ppm) and coupling constants [b] (Hz) of the sugar protons [a].

	H(1')	H(2')	H(3')	H(4')	H(5')	H(5")
1	6.00	4.78	4.34	4.23	3.83	3.76
2	5.90	4.36	4.40	4.10	3.69	3.62
3	5.88	4.78	3.93	4.23	3.75	3.65
	^3J(1',2')	^3J(2',3')	^3J(3',4')	^3J(4',5')	^3J(4',5")	^2J(5',5")
1	6.78	5.21	2.38	2.49	3.25	12.84
2	6.74	5.10	2.42	2.55	3.26	12.92
3 [c]	6.69	5.34	2.54	2.65	3.00	12.85
	(6.76)	(5.27)	(2.36)			

[a] Temp., 298 K; solvent, D_2O; 250 MHz. [b] $|J_{max}| \leq 0.1$ Hz. [c] The values in parenthesis are measured in d_6-DMSO solution.

TABLE 6 Pseudorational parameters and conformation of the (5')hydroxymethyl groups of compounds **1-3** [a,b].

	% S	P_s	ϕ_m	$\%\gamma^+$	$\%\gamma^t$	$\%\gamma^-$
1	82	163.9	30.0	79	21	<1
2	81	165.6	31.1	77.5	21	1.5
3 [c]	82	159.6	28.5	76.5	21	2.5
	(87)	(152.0)	(27.7)			

[a] RMS deviations were found to be 0.00 for all calculations. [b] The values of P_N and ϕ_m for the minor N-conformer were fixed at 11° and 38°, respectively. [c] The data in parenthesis are calculated for d_6-DMSO solution.

TABLE 7 NOE Data [%] of the 1-deazaadenosine derivatives **1-3**.

Compound	Proton irradiated	H(1')	H(2')	*%syn* (±3%)
1	H(8)	10.3	2.5	91
2	H(8)	11.3	3.7	100
3	H(8)	11.0	3.3	97

[a] Temp., 298 K; solvent, d_6-DMSO; 250 MHz.

arises if this hydrogen bond is present in both populated conformations about the exocyclic C(4')-C(5') bond, *viz.*, γ^+ and γ^t (Table 7). As it was pointed out above, there is an excellent correlation between the population of the S-conformers and the γ^+ rotamers (*vide infra*).

The other conformation may be represented by the N-conformer and the γ^t orientation about the exocyclic C(4')-C(5') bond. The use of ball-and-stick models revealed that there are two *syn*-conformations with the (5')CH_2OH···N(3) hydrogen bond. One of them is characterised by the torsion angle χ of *ca.* 65° as it was found in the solid state (*cf.* Fig. 2b) and a very close proximity of a proton of the 5'-OH group and the H(2) and H(2') atoms. The latter was unambiguously confirmed by the corresponding NOEs of 7% and 21% upon irradiation of the 5'-hydroxyl proton. The other *syn*-conformation, as in the case of inosine[14], may result from C(4')-C(5') bond rotation away from the generally favoured γ^+ to the γ^t thereby a proton of the (5')OH group is displaced towards the oxygen atom of the furanose ring. As a consequence the base occupies the *syn*-conformation with a torsion angle χ between 0 and 30°.

It may be remarked that the results of quantum-mechanical calculations predict a stability zone in the *syn*-region of the C-3'-*exo* purine ribonucleosides in which an intramolecular hydrogen bond between the sugar and the base may be formed.[31]

A search for crystallographic nucleoside structures with constrained *syn*-orientation of the base about the glycosyl bond caused by an intramolecular hydrogen bond at the Cambridge Structural Data Base revealed two groups of compounds. The first of them encompasses a large majority of compounds and displays a close steric similarity with **3**, *viz.*, the S-conformation of the sugar moiety, the γ^+ conformation about the C(4')-C(5') bond and a *syn*-conformation with χ = 50-90°. In such a conformation there exists a close proximity between proton of the (5')OH group and H(2') and H(3') protons. Only few compounds have been found to occupy the *syn*-range (χ = 18-40°) accompanied by the N-conformation of the sugar moiety.[32,33] The most interesting feature of these compounds is that they have a reversed type of hydrogen bond, *viz.*, between the 5'-oxygen atom and a proton of the (3)NH group of the base as exemplified by 1-(β-D-arabinofuranosyl)-1*H*-pyrazolo(3,4-*b*)pyridin-4(7*H*)-one[32] and oxoformycin B.[33] In the case of these compounds the (5')OH proton is displaced toward the oxygen atom of the furanose ring, but the γ^+

orientation about the C(4')-C(5') bond essentially remains unchanged. Note in the formycin family there are compounds with both types (N and S) of pentofurnose ring conformation in the solid state.[34] As a whole, these data did not lead to definite conclusions regarding the combination of the main stereochemical parameters for the minor populated conformer of compounds **1-3**.

Inhibitory activity of the 1-deazaadenosine derivatives 2 and 3 against adenosine deaminase

It was previously shown that 1-deazaadenosine (**1**) as well as its 2'-deoxy and 2',3'-dideoxy derivatives are potent inhibitors of adenosine deaminase (ADA; EC 3.5.4.4) from calf intestine.[3,35] It was also suggested that **1** binds to the catalytic site of ADA.[3] Both the 2'- and 3'-hydroxyl groups are not essential for either substrate[36-39] or inhibitor activity[40] of nucleoside derivatives toward ADA. On the contrary, the primary (5')OH group and the *anti*-orientation of the adenine base are of crucial importance for ADA-catalysed deamination of adenosine and its closely related analogues[36,40], including 2',3'-*O*-isopropylidene adenosine.[41] It order to obtain further information about various factors which exert an influence on the inhibitory properties of 1-deazaadenosine, the inhibitory activity of **2** and **3** was studied and compared with that of the parent nucleoside **1**. Compound **2** was found to be the slightly better inhibitor of ADA than the parent **1**. Unexpectedly, the isomer **3** is devoid of any inhibitory activity. These preliminary results demonstrate the importance of the 3'-hydroxyl group of **1** and its 2'-O-methyl derivative **2** during the binding to ADA. Moreover, it is suggested that these compounds bind to ADA at the site which is different from the catalytic center. Detailed investigation of biological properties of 1-dezaadenosine and its O-methyl derivatives are now in progress.

EXPERIMENTAL

General. All compounds were characterised by UV, ^{1}H-, and ^{13}C NMR spectra and were shown to be pure by TLC. ^{1}H NMR Spectra: AC 250 Spectrometer (Bruker, Germany). ^{13}C NMR Spectra: AMX 500 Spectrometer (Bruker, Germany). δ-values in ppm and *J*-values in Hz. The ^3J(H,H) coupling constants for the pseudorotation analysis were simulated and iterated by the WinDaisy program package (Bruker, Germany). UV

Spectra: U-3000 (Hitachi, Japan). Mp.: SMP-20 apparatus (Büchi, Switzerland); not corrected.

7-Amino-3-[(3-*O*-methyl)-β-D-ribofuranosyl]-3*H*-imidazo[4,5-*b*]pyridine (3). A suspension of 1-deazaadenosine (**1**) (500 mg, 2 mmol) in a 10 mM solution (120 ml) of $SnCl_2 \cdot 2H_2O$ was slowly treated with a solution of diazomethane in diethylether. The progress of the reaction was monitored by TLC. When all starting material had disappeared, the reaction was terminated and the mixture was evaporated to dryness. The resulting colourless residue was dissolved in EtOH-H_2O (2:1) (15 ml) and applied to a column (30 × 2 cm) Dowex 1-X2 (OH^-) packed in the same solvent. The faster migrating zone was evaporated to give colourless crystals from MeOH (380 mg, 72%), mp 210-211°C (Found: C, 51.56; H, 5.72; N, 19.91. Calc. for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99%); TLC (silica gel; CH_2Cl_2-MeOH 9:1) 0.30; λ_{max} (MeOH/nm) 258sh, 264 and 280 (ε/dm^3 mol^{-1} cm^{-1} 10300, 12500 and 10100); δ_H [500 MHz; d_6-DMSO] 3.45 (3 H, s, 3'-OMe), 3.46 (2 H, m, 5'-H_2), 3.86 (1 H, m, *J* 5.27, *J* 2.36, 3'-H), 4.53 (1 H, m, 4'-H), 4.68 (1 H, m, *J* 6.42, *J* 6.76, *J* 5.27, 2'-H), 5.42 (1 H, d, *J* 6.42, 2'-OH), 5.89 (1 H, d, *J* 6.76, 1'-H), 6.06 (1 H, dd, *J* 3.4, *J* 8.4, 5'-OH), 6.49 (1 H, d, *J* 5, 6-H), 6.47 (2 H, s, NH_2), 7.80 (1 H, d, *J* 5.5, 5-H), 8.25 (1 H, s, 2-H).

7-Amino-3-[(2-*O*-methyl)-β-D-ribofuranosyl]-3*H*-imidazo[4,5-*b*]pyridine (2). The slower migrating zone yielded compound **2** (110 mg, 20%) as a white foam (Found: C, 51.25; H, 5.84; N, 20.03. Calc. for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99%); TLC (silica gel; CH_2Cl_2-MeOH 9:1) 0.30; λ_{max} (MeOH/nm) 258sh, 263 and 280 (ε/dm^3 mol^{-1} cm^{-1} 10300, 12500 and 9900); δ_H [500 MHz; d_6-DMSO] 3.27 (3 H, s, 2'-OMe), 3.61 (2 H, m, 5'-H_2), 3.99 (1 H, m, 4'-H), 4.29 (1 H, m, 3'-H), 4.44 (1 H, m, 2-H), 5.20 (1 H, d, *J* 4.8, 3'-OH), 5.94 (1 H, dd, *J* 3.7, *J* 8.0, 5'-OH), 6.00 (1 H, d, *J* 6.6, 1'-H), 6.38 (1 H, d, *J* 5.5, 6-H), 6.45 (2 H, s, NH_2), 7.78 (1 H, d, *J* 5.6, 5-H), 8.28 (1 H, s, 2-H).

X-Ray crystal structure. A single crystal (size 0.74 × 0.2 × 0.14 mm) was fixed at the top of a Lindemann capillary with epoxy resin.

Crystal data. $C_{12}H_{16}N_4O_4$. M = 280.29, orthorhombic; space group $P2_12_12_1$ (No. 19), a = 6.834(1), b = 10.600(1), c = 17.586(2) Å, V = 1274.1(3) Å^3, Z = 4, D_x = 1.461 Mg m^{-3}, Mo-Kα radiation (λ = 0.71073 Å), μ = 0.112 mm^{-1}, F(000) = 592, T = 293(2) K.

Data collection and processing. The data were collected on a Siemens P4 four-cycle diffractometer with Mo-Kα radiation and graphite monochromator. A total of 2583 reflections were collected in a range of $2.24° \leq \theta \leq 24.99°$, giving 2217 independent reflections [R(int) = 0.0268]. The data were not corrected for absorption effects.

Solution and refinement. The structure was solved by standard direct methods. Full-matrix least-squares refinements based on F_o^2 were performed with non-hydrogen atoms assigned anisotropic thermal parameters. All hydrogen atoms were found in difference Fourier syntheses, but were constructed in geometrically reasonable positions (bond lengths, bond angles). Especially the planar geometry of the amino group and its orientation relative to the ring atoms was confirmed from difference Fourier synthesis. For all hydrogen atoms a common isotropic thermal parameter was refined.

Programs from the Siemens SHELXTL program package[42] were used for the solution, refinement and graphical representation of the structures. A total of 184 parameters was refined, so that a data/parameter ratio of 12.05 results. The final R_1- and wR_2-values for the data with $I > 2\sigma(I)$ were 0.0387 and 0.0935. Corresponding values for all data were 0.0468 and 0.0986. The goodness-of-fit based on F_o^2 was 1.054. The absolute structure parameter was refined to 0(2), the correct absolute structure could not be determined reliably due to the absence of heavy atoms.

The final difference Fourier map had peak maxima and minima at 0.183 and -0.151 e $Å^{-3}$, without any stereochemical relevance. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).

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