138. Substituent Reactivity and Tautomerism of Isoguanosine and Related Nucleosides

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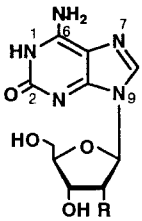
Dedicated to Prof. *David Shugar* on the occasion of his 80th birthday

(3.VII.95)

The substituent reactivity and tautomerism of isoguanine nucleosides is studied. Benzoylation or tosylation of isoguanine nucleosides (pyridine, room temperature) yields the 2-benzoyl derivatives **7c**, **11**, and **12** or the 2-tosyl compounds **13** and **14**. The isobutyrylation of the 6-amino group which did not occur under these conditions was induced in the presence of Me_3SiCl. In the absence of Me_3SiCl, the reactivity of isoguanine substituents decreases in the order from 2-oxo → 5′-OH → 3′-OH → 6-NH_2. From isoguanine nucleosides, the N^1- (**2b**), N^3- (**17**), N^6- (**15a,b**), and 2-*O*-alkylated (**3b**) derivatives were prepared. Their pK_a values were determined and the UV and ^{13}C-NMR spectra compared with regard to the alkylation position. Also the tautomeric forms of isoguanine nucleosides were determined UV-spectrophotometrically in aqueous and nonaqueous solution. Isoguanosine (**1a**), its 2′-deoxy analogue **1b** as well as the N^6-methyl- and 8-substituted derivatives form lactam tautomers in aqueous solution, whereas the lactim form is present in dioxane.

Isoguanosine (isoG; **1a**), its 1-methyl derivate **2a** (doridosine), and the 2-*O*-methylated congener spongosine (**3a**) have been isolated as monomeric nucleosides from various natural sources [1–6]. However, nature has rejected the isoguanine ribo- and 2′-deoxyribonucleosides as constituents of nucleic acids. Nevertheless, the syntheses of isoguanosine (**1a**) [7–9] and 2′-deoxyisoguanosine (**1b**) [10] [11] as well as of oligonucleotides [12–15] and polynucleotides [16] have been carried out. It has been reported that $isoG_d$ (**1b**) forms base pairs with $isoC_d$ as well as with dT [17]. However, compared to $d(A\text{-}T)_6$, the oligomer d[(isoG-T)$_6$] does not show sigmoidal melting [18], and the (A-U-isoG-U)$_3$ duplex is destabilized in comparison to $(A\text{-}U)_6$ [12]. Thus, base pairing between $isoG_d$ and dT is comparably weak. On the other hand, d[(isoG-C)$_3$] shows a cooperative melting profile (T_m 32°, 1M NaCl, 100 mM $MgCl_2$) [18] which demonstrates the existence of an $isoG_d$-dC base pair **A** in a duplex with parallel chain orientation [18] [19].

During the synthesis of the isoguanine-containing oligonucleotides, it became apparent that phosphoramidite chemistry with the 2-*O*-unprotected base is inefficient [20]. It is difficult to incorporate more than one isoG residue into the growing oligonucleotide chain. Our laboratory has employed the diphenylcarbamoyl protecting group [21], others have employed the 4-nitrophenylethyl residue [15] for the protection of the 2-oxo group which improved solid-phase phosphoramidite synthesis. Phosphonate chemistry worked efficiently with 2-*O*-unprotected isoguanine building blocks [12] [13]. As phosphonate coupling uses pivaloyl or adamantanoyl chloride for the condensation reaction, a transient acylation of the 2-oxo group of the isoguanine residue can be considered. Nevertheless, it is also problematic to incorporate several consecutive isoG residues within the



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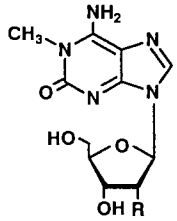
b R = H



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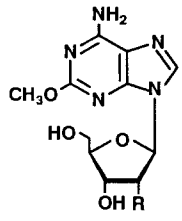
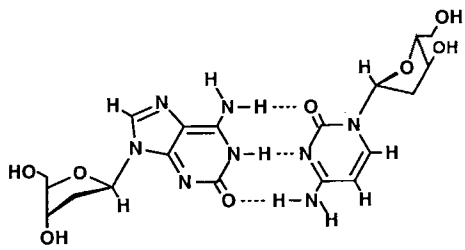


3a R = OH
b R = H



A $isoG_d$-dC
parallel strands

growing oligonucleotide chain. This communication reports on the substituent reactivity of isoguanosine (**1a**) and 2′-deoxyisoguanosine (**1b**) as well as on the tautomerism of related nucleosides.

Results and Discussion. – Isobutyrylation of 2′-deoxyisoguanosine (**1b**) with isobutyryl chloride in pyridine at room temperature yielded the 3′,5′-*O*-diisobutyrylated nucleoside **5** [11] (*Scheme 1*). We obtained the sugar-peracetylated isoguanine nucleosides **4a** and **4b** from **1a** and **1b**, respectively, under the same conditions (62 and 53% yield). The acylation of the exocyclic NH_2 group of **1b**, however, failed. The N^6-isobutyrylation of **1a** (room temperature, pyridine, isobutyryl chloride) was only effective when the protocol of transient protection with trimethylsilyl chloride (Me_3SiCl) [22] was used, giving the N^6-isobutyrylisoguanosine **7a** in 48% yield [14]. Also the N^6-phenoxyacetylated and the N^6-benzoylated derivatives, and a very unusual reversed protecting-group stability were reported [14].

As the results of acylation seem to differ with the various reagents used, we studied isobutyrylation of isoG (**1a**) with isobutyryl chloride at room temperature in the presence [14] and absence of Me_3SiCl. N^6-Isobutyrylisoguanosine (**7a**) was formed indeed when transient silylation was employed, and its structure was confirmed by ^1H- and ^{13}C-NMR spectra (*Table 2*). However, the UV data (*Table 1*) differed from those published earlier [14]. Moreover, the half-life of base deprotection (25% aq. NH_3/EtOH 3:1, 55°) was only 8 min instead of the reported 40 min [14]. We also prepared the N^6-isobutyrylated 2′-deoxyisoguanosine **7b** from **1b** in the presence of Me_3SiCl. Since the N-glycosylic bond of **7b** is very labile, the product was always contaminated with N^6-isobutyrylisoguanine.

Scheme 1

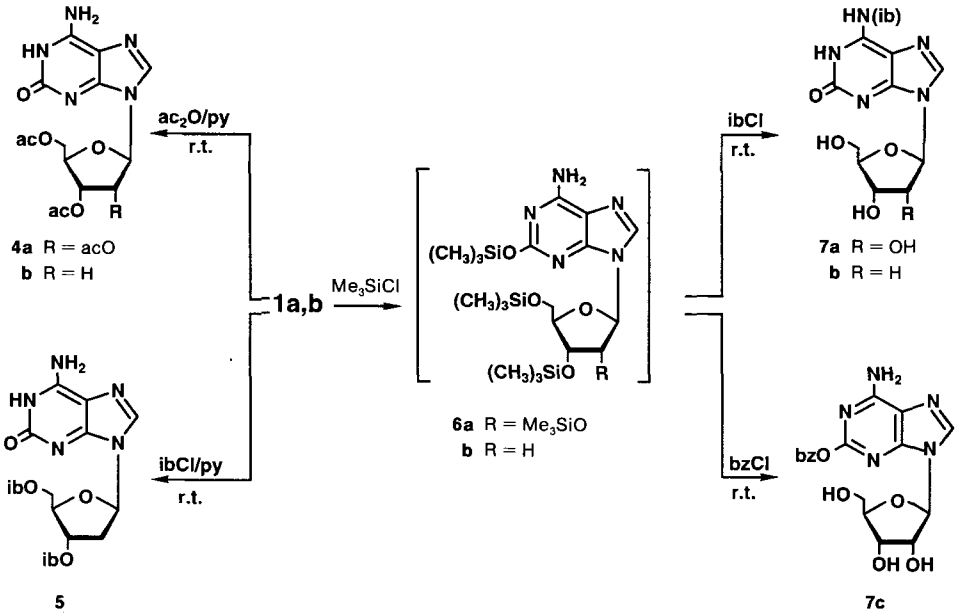


Table 1. *UV Data of Substituted Riboisoguanosine and 2′-Deoxyisoguanosine Derivatives in MeOH*

	λ_{max} (ε) [nm]			λ_{max} (ε) [nm]	
3b	–	267 (12600)	**8**	249 (9000)	298 (9800)
4a	248 (10500)	294 (11100)	**10**	249 (9000)	298 (9900)
4b[a])	248 (7800)	293 (11500)	**11**	231 (31000)	262 (16000)
7a	248 (9200)	332 (12500)	**12**	230 (41900)	261 (15800)
b	248 (8300)	326 (11200)	**13**	226 (17000)	263 (15900)
c	233 (22800)	261 (17700)	**14**	225 (27900)	263 (15300)

[a]) Measured in H_2O/MeOH 5:1.

Table 2. *^{13}C-NMR Chemical Shifts of Isoguanine Nucleosides at 25°* [a])[b])

	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
1a[a])	157.5[c])	152.5[c])	110.6	154.8[c])	140.3	87.0	74.4	71.7	88.9	62.8
a[b]) [12]	158.3[c])	–	109.8	155.9	138.4	87.8	73.0	70.9	85.6	61.7
b[a])	156.7	152.5	109.3	154.1	137.5	83.7	–	71.1	88.1	62.1
2a[b])	153.7	152.2	108.8	151.5	137.9	87.6	72.8	70.7	85.9	61.7
2′a[a])[d]) [33]	157.4	152.3[c])	110.7	152.8[c])	139.1	86.4	74.6	71.7	80.9	64.3
2′aH^{+}[a])[d]) [33]	149.9	140.7	111.8	154.9	138.3	87.8	75.2	71.5	81.9	64.3
2b[b])[e])	153.8	152.1	108.7	151.5	137.6	83.5	–	71.1	88.0	62.0
b[a])	158.7	152.6	110.5	156.5	139.5	84.8	–	71.1	88.4	63.0
3b[a])	162.5	151.2	115.9	157.1	139.4	83.5	–	71.1	87.8	61.9
b[b])[e])	161.7[c])	150.7[c])	115.6	156.8[c])	138.2	84.5	–	71.5	88.2	62.4
4a[b])	157.5	152.1	109.8	155.7	139.4	86.3	73.3	71.0	80.3	63.8
b[a])	157.8	152.2	110.0	155.3	139.1	84.0	36.9	75.3	82.8	64.9

Table 2 (cont.)

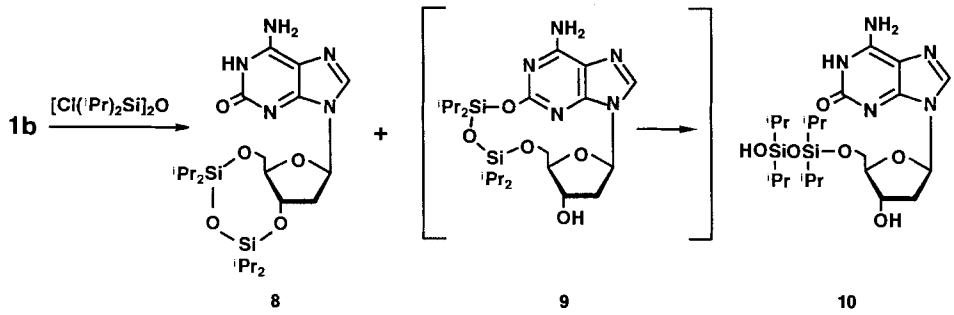
	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
5[b]) [11]	157.1	152.4	109.4	154.6	136.8	82.6	35.6	74.3	81.5	63.6
7a[a])	157.0	152.2	110.0	154.7	139.6	88.3	74.1	71.3	86.6	62.3
c[b])	157.3	150.5	117.9	155.7	140.1	87.4	73.6	70.4	85.7	61.4
10[a])	157.0	152.0	109.2	154.9	137.7	83.2	–	70.9	87.4	63.2
11[b])[e])	157.4	150.4	118.0	156.0	140.0	83.4	–	70.6	84.2	64.6
12[b])	157.5	150.6	118.1	156.1	139.9	83.7	35.8	75.3	81.8	64.4
13)[b])	157.0	149.7	117.8	154.4	140.1	83.6	–	70.8	88.0	61.7
14[b])	157.1	149.5	118.1	154.2	140.5	83.9	–	70.4	84.1	70.3
15a[b]) [25]	158.7	151.2	113.7	154.1	136.2	87.5	73.3	70.5	85.6	61.5
b[b])	158.9	151.4	114.0	154.3	136.2	87.4	73.3	70.5	85.6	61.5
16a[a])	156.7[c])	151.7[c])	110.6	155.4[c])	124.7	90.9[c])	71.0[c])	70.7[c])	87.7[c])	62.2
b[a])	156.9[c])	155.8[c])	106.7	156.8[c])	149.8[c])	89.1[c])	72.2[c])	71.6[c])	87.3[c])	63.4
17[a])	157.7[c])	142.3	114.0	159.0[c])	135.7	93.5[c])	76.4[c])	71.3[c])	84.1[c])	53.2

[a]) (D_6)DMSO/0.4M aq. NH_4OAc. [b]) (D_6)DMSO. [c]) Tentative. [d]) **2′a** stands for the 2′,3′,5′-tri-*O*-acetyl derivative of **2a**. [e]) From gated-decoupled spectra.

Benzoylation of **1a** by the same method gave **7c** (58% yield). The benzoyl group was introduced at the 2-oxo group instead of the 6-amino group [14]. The structure of **7a–c** were confirmed by UV and NMR spectra. According to *Table 1*, the λ_{max} of **7a** is at 332 nm and that of the 2-*O*-benzoyl compound **7c** at 261 nm. The latter value is similar to that of 2′-deoxy-2-methoxyadenosine (**3b**). A similar situation is found for the ^{13}C-NMR spectra (*Table 2*). The downfield shift of C(5) observed for **7c** (8 ppm) with respect to isoguanosine (**1a**) is also found for the 2-methoxy derivative **3b** (6 ppm). As the ^{1}H-NMR data of our benzoyl derivative **7c** are identical with those reported recently for the N^6-benzoyl compound [14], we anticipate that these authors have actually isolated the same material but have reported an improper structure.

We were surprised that there is a difference in the acylation site on isobutyrylation and benzoylation of silylated isoguanosine using various acyl chlorides. The 6-amino group of isoguanine nucleosides is comparably electron-deficient, as it can form a vinologous amide with the 2-oxo substituent. From the experiments carried out with and without silylation, it can be concluded that the isobutyrylation of the 6-amino group of isoguanine nucleosides is the result of a previous silylation reaction occurring at the base moiety. The most probable position of silylation is the O-atom at C(2) which gives an intermediate of type **6** (*Scheme 1*). As a result of the 2-*O*-silylation, the π-system of the base will be reorganized resulting in a more nucleophilic NH_2 group. This NH_2 group is then subjected to isobutyrylation. But it is still surprising that even in the case of silylated isoguanosine **6a**, the 2-*O*-benzoyl derivative **7c** is formed. Apparently, the silyl group can be removed during benzoylation but not during isobutyrylation. The benzoylation of the 2-oxo group deactivates the molecule with the result that the NH_2 group does not react any more. Unfortunately, the silylated intermediates **6a** or **6b** could not be isolated. However, the existence of a 2-*O*-silylated base can be deduced from another experiment: on silylation of 2′-deoxyisoguanosine (**1b**) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane [23], 3′,5′-*O*-bis-silylated derivative **8**, and 5′-*O*-silylated compound **10** were obtained in a 1:1 ratio (*Scheme 2*). It can be considered that **10** was formed *via* intermedi-

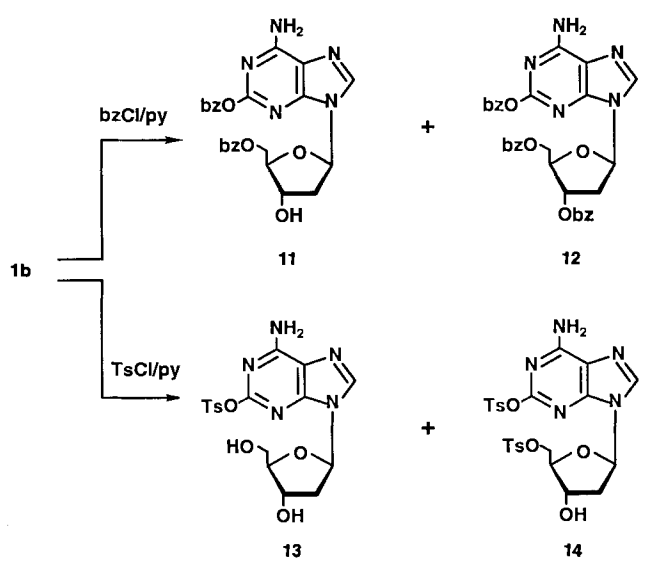
Scheme 2



ate **9**. Compound **9**, which was observed at the beginning of the reaction, showed a UV spectrum similar to that of the methoxy derivative **3b** (TLC scanning) which differed significantly from that of isoguanosine (**1b**; *Table 1*). Apparently, a partial desilylation of **9** by removal of the labile 2-*O*-silyl group then yielded **10**. In the case of the silylation of 2′-deoxyisoinosine with (*tert*-butyl)diphenylsilyl chloride, a 2-*O*-silylated derivative was isolated and characterized [24].

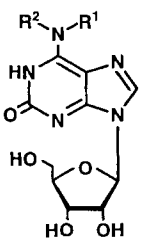
We also studied the benzoylation of 2′-deoxyisoguanosine (**1b**) with benzoyl chloride in the absence of Me_3SiCl. When the ratio of the acylation reagent to the nucleoside was 2.6 (TLC monitoring, silica gel, CH_2Cl_2/MeOH 9:1), a fast-migrating intermediate was formed which was subsequently converted into dibenzoylated **11** (*Scheme 3*, *Table 2*). Both compounds showed UV spectra (TLC scanning and *Table 1*) similar to those of 2′-deoxy-2-methoxyadenosine (**3b**, *Table 1*). If the same reaction was performed with a

Scheme 3

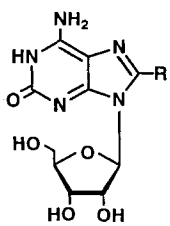


five-fold excess of benzoyl chloride, the tribenzoylated **12** was isolated. According to the ^{13}C-NMR spectra of the dibenzoylated **11** and tribenzoylated **12**, one benzoyl group was at the 2-*O*-position. According to the results discussed above, the reactivity of the OH groups of isoguanine nucleosides decreases in the order 2-OH → 5′-OH → 3′-OH on benzoylation at room temperature in pyridine. The 2-*O*-benzoyl groups of **7c**, **11**, and **12** are comparably labile. Already traces of ammonia led to deprotection. The same type of 2-oxo-group reactivity was observed on tosylation of **1b**: toluene-4-sulfonyl chloride in pyridine afforded the 2-*O*-tosyl derivative **13** together with the 2,5′-*O*-bis-tosyl compound **14** (*Tables 1* and *2*).

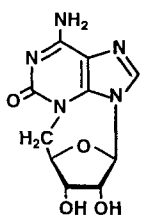
The formation of isoguanine tautomers was already studied on isoguanosine and 9-methylisoguanine derivatives by UV spectroscopy [36]. This phenomenon is expressed by pronounced changes of the UV spectrum which depend on the polarity of solvents. To study the various tautomeric forms of isoguanosine, we prepared derivatives having the H-atoms replaced by alkyl groups thereby fixing the tautomeric states of the base moiety. N^6-Alkyl-substituted derivatives of isoguanosine were previously obtained from corresponding silyl-, thiophenyl-, or thiomethyl derivatives [25] [26]. We used another route starting from 6-*O*-ethylxanthosine [27]; this nucleoside underwent nucleophilic displacement of the ethoxy group by $MeNH_2$ or Me_2NH yielding the derivatives **15a** and **15b**, respectively. The 2′-deoxy-2-methoxyadenosine (**3b**) was obtained from 2-bromo-2′-deoxyadenosine [28] by treatment with NaOMe [29]. Methylation of 2′-deoxyisoguanosine (**1b**) with trimethyl phosphate [30] provided mainly 2′-deoxy-1-methylisoguanosine (= 2′-deoxydoridosine; **2b**; 79%) besides 2′-deoxy-2-methoxyadenosine (**3b**; 20%). Similarly to other purine ribonucleosides, isoguanosine (**1a**) was brominated with elemental Br_2 in AcOH to give the 8-bromo derivative **16a**; bromination in buffered aqueous medium which was used in the case of adenosine or guanosine [31] [32] was not successful due to oxidation. The 8-bromoisoguanosine (**16a**) was then converted with Me_2NH at elevated temperature into 8-dimethylamino derivative **16b**. Reaction of **16a** with thiourea or NaOMe failed.



15a R^1 = H, R^2 = Me
b R^1 = R^2 = Me



16a R = Br
b R = Me_2N



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Many isoguanine nucleosides described above showed only a few of the ^{13}C-NMR signals of their nucleobases if measured in (D_6)DMSO solution. The sugar signals, however, were easily observable. It can be assumed that this is due to tautomerism. This problem was overcome by using a mixture of (D_6)DMSO containing 0.4M aqueous NH_4OAc and employing long relaxation delays (30 s) during acquisition of NMR spectra

[33]. In this case, all compounds gave a full set of their NMR signals (see *Table 2*). Earlier, the ^{13}C-NMR data spin-lattice relaxation time measurement on 1-methylisoguanosine (**2a**) led to the assignment of the ^{13}C-NMR signals, and it was shown that this compound exists as the 6-amino/2-oxo tautomer in aqueous solution [33]. As the δ(C)'s of many isoguanine nucleosides described above are similar to those of **2a** in H_2O-containing solvents (*Table 2*), we followed this assignment for related compounds, *e.g.* for the N^6-methyl- and N^6,N^6-dimethyl derivatives **15a** and **15b**, respectively. As expected, the ^{13}C-NMR spectrum of **17** [34], which can be considered as an N(3)-fixed tautomer, is different from that of the above mentioned Me derivatives. Compared to isoguanosine, the C(6) signal is shifted downfield and that of C(4) upfield; the signal of C(2) is almost unaffected as it is adjacent to a substituted N-atom (N(3)) like in **2a** (Me–N(1)). This excludes a 3*H*-tautomer structure for isoguanine nucleosides. As found for guanosine N(3) of **2a** and not N(7) was determined as position of protonation [33]. Strong upfield shifts of the δ's C(2) and C(4) relative to the values of **2a** are an unequivocal indication of protonation at N(3) (*Table 2*). Compounds **11**–**14** carrying a benzoyl or a tosyl group at the 2-*O*-position show very similar ^{13}C-NMR chemical shifts for the base moiety as the methoxy nucleoside **3b**.

The UV spectra of N^6-substituted isoguanosine derivatives **15a**,**b** show a small bathochromic shift compared to the parent compounds **1a**,**b** (*Table 3*). The same was observed for the 8-bromo-substituted compound **16a**. The Me substitution at N(1) does not influence the character of the UV spectrum (see **1b** and **2b** in *Fig. 1a*). In contrary, the spectrum of **17** which could be considered as a 3*H*-fixed tautomer differs significantly from **1a** or **1b** and from **2b**, and the UV spectrum of 2'-deoxy-2-methoxyadenosine (**3b**) has a completely different character (*Fig. 1, a*). Comparison of the UV and the ^{13}C-NMR

Table 3. *UV Data and p*K *Values of Isoguanine Ribonucleosides and 2'-Deoxyribonucleosides*[a])

	pH 1.0 $\lambda_{max}(\varepsilon)$ [nm]	pH 7.0 $\lambda_{max}(\varepsilon)$ [nm]	pH 12.0 $\lambda_{max}(\varepsilon)$ [nm]	p*K* Value
1a	235 (6100)	247 (8900)	251 (6900)	3.4
	283 (12700)	293 (11100)	285 (1060)	9.8
b	235 (5200)	247 (9100)	249 (6600)	3.45
	284 (11500)	292 (10100)	284 (9800)	9.80
2b	235 (5400)	249 (7800)	249 (7800)	
	283 (12500)	292 (11300)	292 (11300)	3.45
3b	249 (9900)	267 (13700)	267 (13700)	3.20
15a	283 (12900)	249 (7800)	254 (5800)	3.55
	–	292 (10300)	284 (11300)	9.65
b	288 (15800)	251 (9900)	230 (15800)	3.55
	–	299 (13300)	263 (8100)	9.70
	–	–	288 (13600)	
16a	242 (7400)	254 (8700)	260 (7200)	3.30
	288 (12400)	297 (10700)	289 (10900)	9.45
b	318 (21500)	258 (12600)	260 (10500)	1.35
	–	306 (11000)	292 (11200)	3.80
				9.65
17	238 (5500)	279 (11500)	279 (11500)	3.95
	286 (13100)	–	–	

[a]) Measured in aqueous solution.

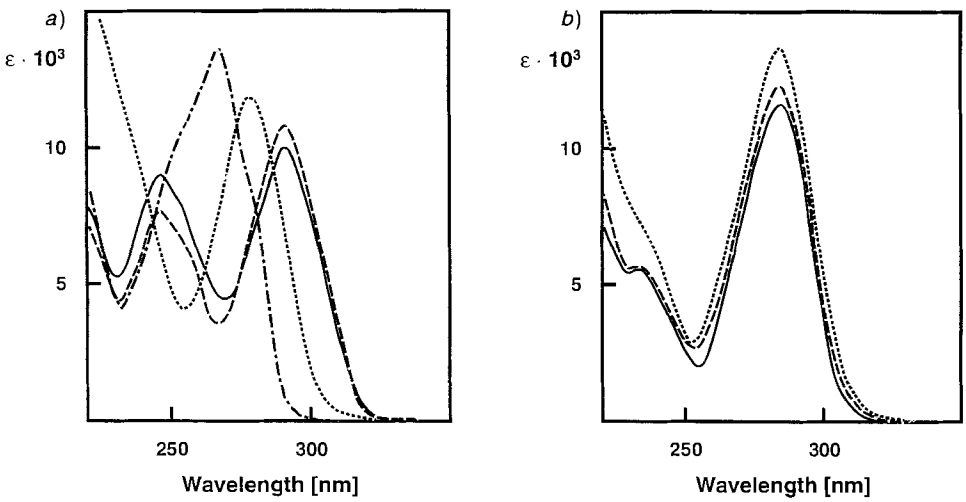
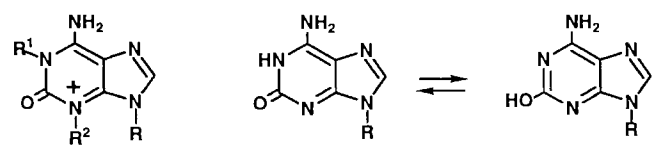


Fig. 1. *UV Spectra of* a) *unprotonated* **1b** (——), **2b** (– –), **17** (---), *and* **3b** (–·–) *in* H_2O *at pH 7.0; and of* b) *cationic forms of* **1b** (——), **2b**, (– –), *and* **17** (----) *in* H_2O *at pH 1.0*

data of the above mentioned compounds confirmed that – apart from the 2-*O*-substituted derivatives – the isoguanine nucleosides exist in neutral aqueous phase as 1*H*/6-amino/2-oxo tautomers.

Isoguanosine (**1a**) has two p*K* values, the lower one corresponds to protonation, the higher one to deprotonation of the base. The substituents of the functional group or the ring N-atoms do not distinctly influence the p*K* values. However, it was observed that small changes are induced by the variation of the substituents. As found by ^{1}H-NMR spin-lattice lifetime measurements [33], quantum-mechanical calculation, and crystallographic data, N(3) was established as protonation site [35]. The similarity of the UV spectra of the cationic forms of 2′-deoxyisoguanosine (**1b**), 2′-deoxydoridosine (**2b**), and



18 $R^1 = R^2 = H$ (from **1**)
R^1 = Me, R^2 = H (from **2**)
R^1 = H, $R^2 = -CH_2-$
(from **17**)

19a
R = sugar residue

19b

also of the cyclo derivative **17** (*Fig. 1b*) show that these cationic forms are isoionic (see **18**).

Isoguanine nucleosides are very sensitive towards solvent-polarity changes which was already observed on similar nucleobase alkyl derivatives [36]. *Fig. 2, a*, shows the UV

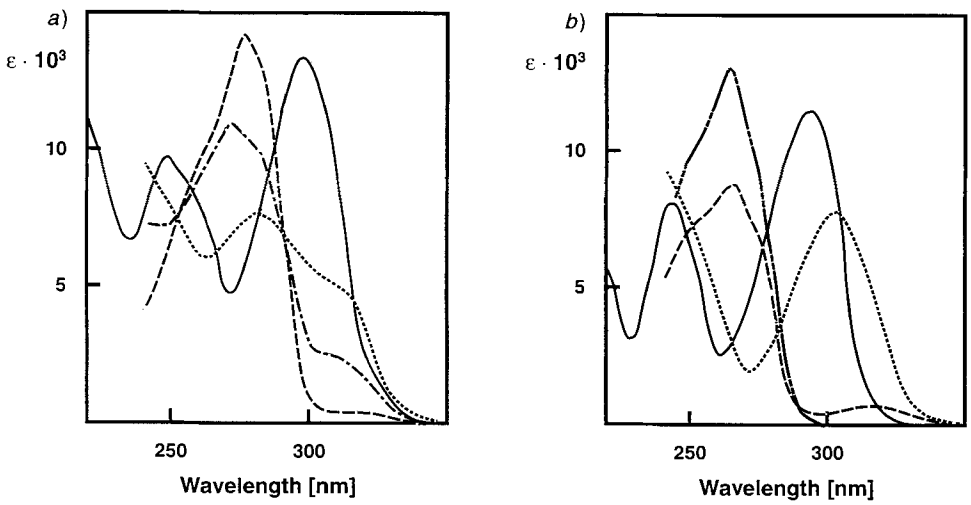


Fig. 2. UV Spectra of a) *the neutral molecule* **15b** *in* H_2O (pH 7.0) (——), *in anhydrous dioxane* (– –), *in dioxane/*H_2O *97:3* (–·–), *and dioxane/*H_2O *88:12* (---) *and of* b) *the neutral molecule* **4b** *in* H_2O*/MeOH 5:1* (——), *in anhydrous dioxane* (– –), *in dioxane/*H_2O *91:9* (---), *and* **3b** *in anhydrous dioxane* (– –)

spectrum of N^6,N^6-dimethylisoguanosine (**15b**) in anhydrous dioxane which was used as a nonpolar medium. Compared to the UV spectrum in H_2O, the maximum at 299 nm is missing, whereas a new one appears at 270 nm. Addition of small amounts of H_2O changes the spectrum of **15b** dramatically. *Fig. 2b* shows a comparison of the UV spectrum of compound **4b** (2′-deoxyisoguanosine (**1b**) is not soluble enough in dioxane) in dioxane with the spectrum of 2′-deoxy-2-methoxyadenosine (**3b**) which could be considered as a fixed lactim form. The addition of H_2O to the dioxane solution also induces significant changes of the UV spectrum of **4b**. The changes do not depend on the substitution of the amino group. It should be noted that doridosine (**2a**) or the 2′-deoxy derivative **2b** do not exhibit such phenomena as their UV spectra taken in several solvents do not differ significantly. From these findings a tautomeric lactim-lactam equilibrium occurring on isoguanosine is also observed on the 2′-deoxy- (**1b**), the 8-bromo derivatives (**16a**) as well as on compounds substituted at the exocyclic amino group (**15a, b**). The lactim form **19b** exists predominantly in nonpolar solvents, whereas the lactam form **19a** is formed in water. It stays to prove if the ease of the prototropic shift as in the case of isoguanine nucleosides can cause mutagenic events during replication.

We thank Dr. *H. Rosemeyer* for the NMR measurements, Mr. *T. Wenzel* for the preparation of computer drawings, and Mrs. *A. Lippus* for her help during the preparation of the manuscript. Financial support by the *Bundesministerium für Bildung und Forschung* is gratefully acknowledged.

Experimental Part

General. See [37]. TLC: Glass plates coated with a 0.25 mm layer of silica gel *Sil G-25* with fluorescent indicator UV_{254} (*Merck*, Germany). TLC Scanning: *CS-930* TLC scanner (*Shimadzu*, Japan). Column flash chromatography (FC): silica gel *60H* at 0.5 bar. Hydrophobic resin: *Serdolit AD-4*, 0.1–0.2 mm (*Serva*, Germany). Half-life values: measured on *U-3200* spectrophotometer (*Hitachi*, Japan) connected with a temperature controller (*Lauda*, Germany). M.p. (not corrected): *Büchi-SMP-20* apparatus (*Büchi*, Switzerland). p*K* values: determined at r.t. in *Teorell-Stenhagen* buffer. UV Spectra: λ_{max} in nm, ε in $M^{-1}cm^{-1}$. NMR Spectra: *AC-250* and *AMX-500* spectrometer (*Bruker*, Germany); δ values in ppm downfield from internal $SiMe_4$. Elemental analyses: *Mikroanalytisches Laboratorium Beller*, Göttingen, Germany.

*6-Amino-9-(2-deoxy-β-*D-erythro-*pentofuranosyl)-1,9-dihydro-1-methyl-2*H*-purin-2-one* (= *2′-Deoxydoridosine*; **2b**). To the suspension of 2′-deoxyisoguanosine (**1b**; 267 mg, 1 mmol) [10] in H_2O (4.0 ml), trimethyl phosphate (4.2 ml, 36 mmol) was added. The mixture was stirred at 50° and kept alkaline by addition of 1M NaOH. After 24 h, the mixture was neutralized and evaporated several times with MeOH to give an oil. The residue was dissolved in EtOH, adsorbed on silica gel, and applied on the top of a silica-gel column (2 × 10 cm). Elution with CH_2Cl_2/MeOH 9:1 to 3:7 (300 ml) gave first **3b** (56 mg, 20%; see below) and then **2b** as colourless needles from H_2O (222 mg, 79%). TLC (i-PrOH/25% aq. NH_3 soln./H_2O 7:1:2): R_f 0.6. M.p. 213–216° (dec.). ^{1}H-NMR ((D_6)DMSO): 2.12, 2.59 (2*m*, 2 H–C(2′)); 3.33 (*s*, Me); 3.48 (*m*, 2 H–C(5′)); 3.83 (*s*, H–C(4′)); 4.34 (*s*, H–C(3′)); 5.23 (br. *s*, OH–C(3′), OH–C(5′)); 6.08 (‘*t*’, *J* = 6.6, H–C(1′)); 7.92 (*s*, H–C(8)); 8.12 (br. *s*, NH_2). Anal. calc. for $C_{11}H_{15}N_5O_4$ (281.3): C 46.97, H 5.38, N 24.90; found: C 47.05, H 5.36, N 24.92.

2′-Deoxy-2-methoxyadenosine (**3b**). A soln. of 2-bromo-2′-deoxyadenosine [28] (500 mg, 1.5 mmol) in MeOH (6 ml) containing 1M NaOMe (4 ml) was heated under reflux for 4 h. The mixture was evaporated, the residue chromatographed (silica gel *60H* (1 × 9 cm), CH_2Cl_2 (100 ml), then CH_2Cl_2/MeOH (9:1)), and the product crystallized from EtOH: needles (360 mg, 85%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.3. M.p. 170–171° ([29]: 173–174.5°). ^{1}H-NMR ((D_6)DMSO): 2.50, 2.75 (2*m*, 2 H–C(2′)); 3.60 (*m*, 2 H–C(5′)); 3.83 (*s*, MeO); 3.87 (*s*, H–C(4′)); 4.35 (*s*, H–C(3′)); 5.02 (*t*, *J* = 5.3, OH–C(5′)); 5.29 (*d*, *J* = 3.3, OH–C(3′)); 6.27 (*t*, *J* = 6.8, H–C(1′)); 7.27 (*s*, NH_2)); 8.14 (*s*, H–C(8)).

*2′,3′,5′-Tri-*O*-acetylisoguanosine* (**4a**). To the stirred soln. of isoguanosine (**1a**; 570 mg, 2.1 mmol) in anh. pyridine (20 ml), Ac_2O (6 ml, 63.6 mmol) was added. Within 1 h, **1a** was dissolved and stirring continued at r.t. for 1 h. Under cooling (ice bath), MeOH (2 ml) was added, the mixture evaporated and then co-evaporated twice with EtOH, the residue chromatographed (silica gel (2 × 20 cm), CH_2Cl_2 (200 ml), then CH_2Cl_2/MeOH 9:1), and the product crystallized from EtOH: colourless needles (545 mg, 62%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.3. M.p. 103–105°. ^{1}H-NMR ((D_6)DMSO/D_2O): 1.98, 2.04 (2*s*, 3 Me); 3.60 (*m*, 2 H–C(5′)); 3.97 (*s*, H–C(4′)); 4.17 (*m*, H–C(3′)); 4.93 (*t*, *J* = 5.9, H–C(2′)); 6.57 (*d*, *J* = 7.4, H–C(1′)); 7.88 (*s*, H–C(8)). Anal. calc. for $C_{16}H_{19}N_5O_8$ (409.36): C 46.95, H 4.68, N 17.11; found: C 47.01, H 4.78, N 17.17.

*3′,5′-Di-*O*-acetyl-2′-deoxyisoguanosine* (**4b**). To the stirred soln. of 2′-deoxyisoguanosine (**1b**; 260 mg, 0.97 mmol) in pyridine (15 ml), Ac_2O (0.7 ml, 7 mmol) was added. The mixture was stirred at r.t. overnight. Then, Ac_2O was hydrolyzed with MeOH (0.3 ml, ice bath), the mixture evaporated and co-evaporated twice with toluene, the residue chromtographed (silica gel (3 × 12 cm), $CHCl_3$ (200 ml), then $CHCl_3$/MeOH 8:2), and the product crystallized from EtOH: colourless needles (180 mg, 53%). TLC ($CHCl_3$/MeOH 9:1): R_f 0.2. M.p. 235–237°. ^{1}H-NMR ((D_6)DMSO): 2.03, 2.08 (2*s*, 2 Me); 2.40, 2.93 (2*m*, 2 H–C(2′)); 4.25 (*m*, H–C(4′), 2 H–C(5′)); 5.29 (*m*, H–C(3′)); 6.11 (‘*t*’, *J* = 7.1, H–C(1′)); 7.55 (br. *s*, NH_2); 7.96 (*s*, H–C(8)); 10.56 (br. *s*, H–N(1)). Anal. calc. for $C_{14}H_{17}N_5O_6$ (351.32): C 47.86, H 4.88, N 19.93; found: C 48.06, H 4.76, N 19.78.

*6-Amino-2-(benzoyloxy)-9-(β-*D*-ribofuranosyl)-9*H*-purine* (**7c**). To the suspension of **1a** (150 mg, 0.53 mmol) in dry pyridine (10 ml), Me_3SiCl (0.6 ml, 5.0 mmol) was added under stirring at r.t. Stirring was continued for 1.5 h, then benzoyl chloride (0.15 ml, 1.27 mmol) was introduced. After 2 h, H_2O (0.6 ml) was added at 0°, followed by 25% aq. NH_3 soln. (0.21 ml). A precipitate was removed by filtration, and more H_2O (10 ml) was added. The soln. was extracted with AcOEt (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue applied to FC (column 4 × 10 cm, CH_2Cl_2/MeOH 98:2 → 80:20 (300 ml)): colourless solid (120 mg, 58%). TLC (CH_2Cl_2/MeOH 8:2): R_f 0.36. ^{1}H-NMR ((D_6)DMSO): 3.54, 3.65 (2*m*, 2 H–C(5′)); 3.95 (*d*, H–C(4′)); 4.13 (*m*, H–C(3′)); 4.56 (*m*, H–C(2′)); 5.11 (*t*, *J* = 5.2, OH–C(5′)); 5.17 (*d*, *J* = 4.9, OH–C(3′)); 5.47 (*d*, *J* = 6.2, OH–C(2′)); 5.85 (*d*, *J* = 6.0, H–C(1′)); 7.65 (*t*, 2 H_m); 7.77–7.80 (*m*, H_p, NH_2); 8.12 (*d*, 2 H_o); 8.43 (*s*, H–C(8)).

Reaction of **1b** *with 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane.* To the suspension of **1b** (267 mg, 1 mmol) in dry pyridine (8.0 ml), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.36 ml, 1.1 mmol) was added under stirring. Stirring was continued for 24 h at r.t. and then the mixture concentrated to 2 ml, poured into ice-water (50 ml), and extracted with CH_2Cl_2 (3 × 5 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the residue

applied to FC (column 2 × 10 cm, CH_2Cl_2/MeOH 95:5 (100 ml) 9:1 (100 ml), and 8:2 (50 ml)): **8** (168 mg, 33%) and then **10** (165 mg, 31%).

*6-Amino-9-[2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-*erythro*-pentofuranosyl]-1,9-dihydro-2*H*-purin-2-one* (**8**): colourless powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.3. ^{1}H-NMR ((D_6)DMSO): 0.92–1.19 (*m*, 4 ^{i}Pr); 2.45, 2.67 (2*m*, 2 H–C(2′)); 3.78 (*s*, H–C(4′)); 3.95 (*m*, 2 H–C(5′)); 4.81 (*m*, H–C(3′)); 6.07 (‘*t*’, J = 3.5, H–C(1′)); 7.66 (br. *s*, NH_2); 7.86 (*s*, H–C(8)); 10.74 (br. *s*, H–N(1)). Anal. calc. for $C_{22}H_{39}N_5O_5Si_2$ (509.76): C 51.84, H 7.71, N 13.74; found: C 51.96, H 7.68, N 13.54.

*6-Amino-9-[2-deoxy-5-*O*-(3-hydroxy-1,1,3,3-tetraisopropyldisiloxan-1-yl)-β-*D*-*erythro*-pentofuranosyl]-1,9-dihydro-2*H*-purin-2-one* (**10**): colourless powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.3. ^{1}H-NMR ((D_6)DMSO): 0.80–1.03 (*m*, 4 ^{i}Pr); 2.22 (*m*, 2 H–C(2′)); 3.82–3.86 (*m*, 2 H–C(5′), H–C(4′)); 4.36 (*m*, H–C(3′)); 5.29 (br. *s*, OH–C(3′)); 6.11 (‘*t*’, J = 6.4, H–C(1′)); 7.68 (br. *s*, NH_2); 7.86 (*s*, H–C(8)); 10.72 (br. *s*, H–N(1)). Anal. calc. for $C_{22}H_{41}N_5O_6Si_2$ (527.77): C 50.07, H 7.83, N 13.27; found: C 49.98, H 7.86, N 13.20.

*6-Amino-9-(5-*O*-benzoyl-2-deoxy-β-*D*-erythro-pentofuranosyl)-2-(benzoyloxy)-9*H*-purine* (**11**). To the stirred suspension of **1b** (268 mg, 1 mmol) in pyridine (3 ml), benzoyl chloride (0.3 ml, 2.6 mmol) was added and stirring continued at r.t. for 9 h. The mixture was poured into ice-water and the white precipitate filtered off, washed with H_2O and AcOEt, and dried: colourless solid (230 mg, 48%). An additional amount (65 mg) was obtained by evaporation of the filtrates, dissolving of the residue in MeOH, and precipitation with H_2O total yield: 295 mg (62%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.5. M.p. 137–139° (dec.). ^{1}H-NMR ((D_6)DMSO): 2.43, 2.81 (2*m*, 2 H–C(2′)); 4.14–4.56 (*m*, H–C(3′), H–C(4′), 2 H–C(5′)); 5.52 (br. *s*, OH–C(3′)); 6.34 (‘*t*’, J = 6.1, H–C(1′)); 7.45–8.10 (*m*, arom. H); 7.74 (*s*, NH_2); 8.35 (*s*, H–C(8)). Anal. calc. for $C_{24}H_{21}N_5O_6$ (475.46): C 60.63, H 4.45, N 14.73; found: C 60.69, H 4.55, N 14.83.

*6-Amino-2-(benzoyloxy)-9-(3,5-di-*O*-benzoyl-2-deoxy-β-*D*-erythro-pentofuranosyl)-9*H*-purine* (**12**). To the stirred suspension of **1b** (267 mg, 1 mmol) in pyridine (3 ml), benzoyl chloride (0.3 ml, 2.6 mmol) was added. The mixture was stirred at r.t. for 20 min. Then, a second portion of benzoyl chloride (0.4 ml, 3.4 mmol) was added and stirring continued for 2 h. The mixture was poured into ice-water and extracted with CH_2Cl_2 (4 × 10 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue chromatographed (silica gel (2 × 15 cm), CH_2Cl_2/MeOH 99:1 → 97:3 (300 ml)): colourless foam (281 mg, 49%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.9. ^{1}H-NMR ((D_6)DMSO): 2.79, 3.23 (2*m*, 2 H–C(2′)); 4.60 (*m*, 2 H–C(5′), H–C(4′)); 5.77 (*m*, H–C(3′)); 6.49 (‘*t*’, J = 6.9, H–C(1′)); 7.45–8.10 (*m*, arom. H); 7.79 (br. *s*, NH_2); 8.41 (*s*, H–C(8)). Anal. calc. for $C_{31}H_{25}N_5O_7$ (579.57): C 64.24, H 4.35, N 12.08; found: C 64.49, H 4.39, N 11.80.

Tosylation of **1b**. To the stirred suspension of **1b** (134 mg, 0.5 mmol) in dry pyridine (2.5 ml), TsCl (96 mg, 0.5 mmol) was added. After 4 h stirring an additional portion of TsCl (96 mg, 0.5 mmol) was added. After 1h, the mixture was poured into ice-water (15 ml) and extracted with CH_2Cl_2 (4 × 5 ml). The combined org. phase was washed with 5% aq. $NaHCO_3$ soln. (2 × 5 ml) and H_2O (2 × 10 ml), dried (Na_2SO_4), and evaporated and the residue chromatographed (silica gel (2 × 10 cm), CH_2Cl_2 (100 ml), then CH_2Cl_2/MeOH 95:5 (200 ml) and 9:1 (200 ml)): **14** (40 mg, 14%) and then **13** (97 mg, 46%).

*6-Amino-9-(2-deoxy-β-*D*-erythro-pentofuranosyl)-2-*O*-[(tol-4-yl)sulfonyloxy]-9*H*-purine* (**13**): colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.3. ^{1}H-NMR ((D_6)DMSO): 2.19, 2.54 (2*m*, 2 H–C(2′)); 2.42 (*s*, Me); 3.45 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.31 (*m*, H–C(3′)); 4.89 (*t*, J = 5.4, OH–C(5′)); 5.31 (*d*, J = 4.0, OH–C(3′)); 6.10 (‘*t*’, J = 6.7, H–C(1′)); 7.45–8.00 (*m*, NH_2, arom. H); 8.32 (*s*, H–C(8)). Anal. calc. for $C_{17}H_{19}N_5O_6S$ (421.43): C 48.45, H 4.54, N 16.62, S 7.61; found: C 48.27, H 4.62, N 16.80, S 7.43.

*6-Amino-9-{2-deoxy-5-*O*-[(tol-4-yl)sulfonyl]-β-*D*-erythro-pentofuranosyl}-2-*O*-[(tol-4-yl)sulfonyloxy]-9*H*-purine* (**14**): colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.6. ^{1}H-NMR ((D_6)DMSO): 2.18, 2.54 (2*m*, 2 H–C(2′)); 2.33, 2.41 (2*s*, 2 Me); 4.02 (*m*, 2 H–C(5′)); 4.14 (*m*, H–C(4′)); 4.26 (*m*, H–C(3′)); 5.49 (*d*, J = 4.1, OH–C(3′)); 6.09 (‘*t*’, J = 6.5, H–C(1′)); 7.26–7.92 (*m*, NH_2, arom. H); 8.17 (*s*, H–C(8)). Anal. calc. for $C_{24}H_{25}N_5O_8S_2$ (575.62): C 50.08, H 4.38, N 12.17, S 11.14; found: C 50.01, H 4.29, N 11.94, S 10.86.

N^6-*Methylisoguanosine* (**15a**). The soln. of 6-*O*-ethylxanthosine [27] (400 mg, 1.3 mmol) in 33% $MeNH_2$/MeOH (20 ml) was heated in a steel vessel at 100° for 8 h. The mixture was evaporated and the residue crystallized from anh. EtOH: colourless needles (290 mg, 76%). TLC (silica gel, $CHCl_3$/MeOH/AcOH 50:10:5): R_f 0.3. M.p. 188–190° (dec.; [25]: 188–190°). Anal. calc. for $C_{11}H_{15}N_5O_5$ (297.27): C 44.44, H 5.09, N 23.56; found: C 44.62, H 5.09, N 23.41.

N^6,N^6-*Dimethylisoguanosine* (**15b**). As described for **15a**, with 6-*O*-ethylxanthosine (625 mg, 2.0 mmol) and 30% Me_2NH/MeOH (30 ml): colourless needles (470 mg, 75%). TLC (silica gel, $CHCl_3$/MeOH/AcOH 50:10:5): R_f 0.3. M.p. 184–186° (dec. [25]: 230–232°). Anal. calc. for $C_{12}H_{17}N_5O_5$ (311.30): C 46.30, H 5.50, N 22.50; found: C 46.25, H 5.62, N 22.37.

8-Bromoisoguanosine (**16a**). To a suspension of **1a** (660 mg, 2.3 mmol) [7] in AcOH (20 ml) containing anh. NaOAc (495 mg, 6 mmol) Br_2 (560 mg, 3 mmol) in AcOH (3 ml) was added during 1 h. The mixture was stirred at r.t. for 4 h and then evaporated, the residue co-evaporated twice with toluene/EtOH (depurination was observed in the presence of H_2O), the residue treated with a small volume of H_2O, and the chromatographically pure material filtered (660 mg, 78%). A small sample was crystallized from EtOH/H_2O: needles. TLC (silica gel, $CHCl_3$/MeOH/AcOH 50:10:5): R_f 0.25. M.p. > 250° (dec.). ^{1}H-NMR ((D$_6$)DMSO/D_2O): 3.50–3.65 (*m*, 2 H–C(5′)); 4.00 (*s*, H–C(4′)); 4.15 (*d*, *J* = 3.9, H–C(3′)); 4.88 (*t*, *J* = 5.9, H–C(2′)); 5.68 (*d*, *J* = 7.1, H–C(1′)). Anal. calc. for $C_{10}H_{12}BrN_5O_5$ (362.2): C 33.17, H 3.34, N 19.34; found: C 33.30, H 3.27, N 19.52.

8-(Dimethylamino)isoguanosine (**16b**). As described for **15a**, with **16a** (230 mg, 0.64 mmol) and 30% Me_2NH/MeOH (4 ml). Crystallization from EtOH/AcOEt gave colourless needles (130 mg, 63%). TLC (silica gel, $CHCl_3$/MeOH/AcOH 50:10:5): R_f 0.3. M.p. > 215°. ^{1}H-NMR ((D$_6$)DMSO/D_2O): 2.75 (*s*, 2 Me); 3.50–3.65 (*m*, 2 H–C(5′)); 3.96 (*s*, H–C(4′)); 4.17 (*d*, *J* = 4.4, H–C(3′)); 4.93 (*t*, *J* = 6.2, H–C(2′)); 5.58 (*d*, *J* = 7.2, H–C(1′)). Anal. calc. for $C_{12}H_{18}N_6O_5$ (326.3): C 44.17, H 5.56, N 25.75; found: C 44.10, H 5.68, N 25.90.

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