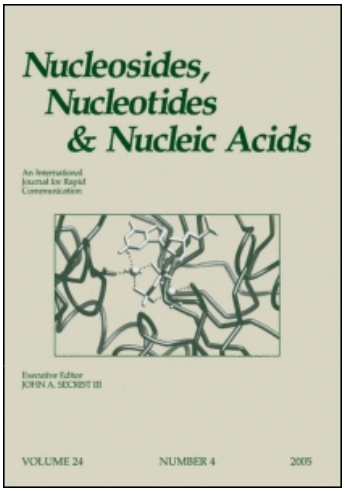

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Synthesis and ^{1}H and ^{13}C NMR Spectral Characteristics of 8-Bromo-2',3'-Dideoxyguanosine and 8-Bromo-2',3'-Dideoxyinosine

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SYNTHESIS AND ^{1}H AND ^{13}C NMR SPECTRAL CHARACTERISTICS OF 8-BROMO-2',3'-DIDEOXYGUANOSINE AND 8-BROMO-2',3'-DIDEOXYINOSINE[1]

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ABSTRACT: Reaction of 2',3'-dideoxyguanosine **1a** as 5'-O-*tert*-butyldimethylsilyl derivative **1b** and 2',3'-dideoxyinosine **2a** with bromine in acetate buffer conducted in heterogeneous medium afforded 8-bromo-2',3'-dideoxyguanosine **3a** (44% after deprotection) and 8-bromo-2',3'-dideoxyinosine **4a** (12%) respectively. The change of conformational preferences anti → syn on 8-bromo substitution of 2',3'-dideoxynucleosides is reflected in the ^{1}H and ^{13}C NMR spectra by characteristic shifts of H-2', H-3', C-2' and C-3' signals.

INTRODUCTION

2',3'-Dideoxynucleosides: 3'-azido-3'-deoxythymidine (AZT, zidovudine), 2',3'-dideoxyinosine (ddI, didanosine) and 2',3'-dideoxycytidine (ddC, zalcitabine), inhibitors of human immunodeficiency virus (HIV) reverse transcriptase are the first antiretroviral agents approved for the treatment of HIV infection.[2] There is therefore a considerable interest in the synthesis of their analogues aimed at establishing structure-activity relationships for the members of 2',3'-dideoxynucleoside family and improving their chemotherapeutic properties.

We present here the synthesis of new analogues of anti-HIV agents: 2',3'-dideoxyguanosine (**1a**, ddG) and 2',3'-dideoxyinosine (**2a**) namely 8-bromo-2',3'-dideoxyguanosine (**3a**, $Br^{8}ddG$) and 8-bromo-2',3'-dideoxyinosine (**4a**, $Br^{8}ddI$).

Presumed dominance of the syn conformation[3,4] in the structure of these analogues may be expected to alleviate one of the shortcomings of the parent

dideoxynucleosides - their strong susceptibility to catabolic phosphorolysis caused by the action of purine nucleoside phosphorylase (PNP).[5,6] Earlier studies on the structure-activity relationship for substrate and inhibitor properties towards PNP revealed that the substrate should occur in the anti conformation.[7] In addition, bromo substituents may also improve lipophilic properties of the parent compounds **1a** and **2a** and enhance their penetration potential into the central nervous system.[8]

RESULTS AND DISCUSSION

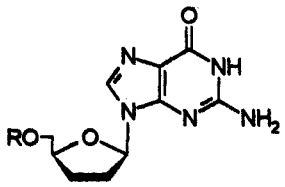
Synthesis

For preparing ddG and ddI to be used as starting materials for 8-bromo derivatives we introduced some modifications which significantly improved known procedures[9-11] in terms of simplicity and yield.

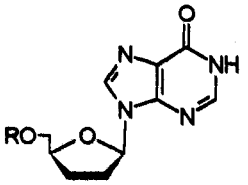
Thus our route towards **1a** employed silylation for 5'-OH protection of starting 2'-deoxyguanosine (**5a**); on application of literature procedure[12] 5'-O-(*tert*-butyldimethylsilyl-2'-deoxyguanosine (**5b**) was obtained in 82% yield. The protected compound **5b** was subsequently converted into corresponding 3'-O-(imidazol-1-yl)thiocarbonyl derivative (**5c**) in 75% yield, deoxygenated with tri-*n*-butyltin hydride/AIBN to **1b** in 91%. In all the stages, pure compounds were obtained without recourse to chromatography. The product **1b** was then deprotected with tetra-*n*-butylammonium fluoride to give **1a** in 77% yield.

Aiming towards **2a**, we successfully challenged the known acid instability of the glycosidic bond of 2',3'-dideoxynucleosides[13,14] by using acid-labile protecting group (dimethoxytrityl) for 5'-OH protection of starting 2'-deoxyinosine (**5d**). 5'-O-(4,4'-Dimethoxytrityl)-2'-deoxyinosine (**5e**)[15] was converted into thioimidazolide (**5f**) and then deoxygenated with tri-*n*-butyltin hydride/AIBN to yield 5'-O-dimethoxytrityl-2',3'-dideoxyinosine **2b** in 84%. 5'-O-Detritylation of **2b** with 80% acetic acid at 0°C for 1.5 h was followed by careful adjustment to pH 9.5 with aqueous ammonia. Chromatographic purification and crystallization afforded **2a** in 65% yield. It is noteworthy that under the above conditions the glycosidic bond underwent hydrolysis to a very small extent.

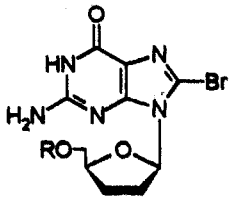
We applied a commonly used bromination method of 2'-deoxyguanosine **5a**[16] to its 2',3'-dideoxy congener **1a**. Bearing in mind the enhanced instability of the



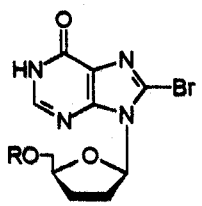
1a; R = H
b; R = TBDMS



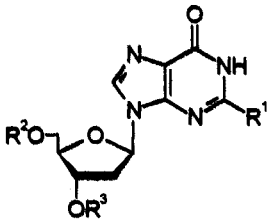
2a; R = H
b; R = DMTr
c; R = TBDMS
d; R = Ac



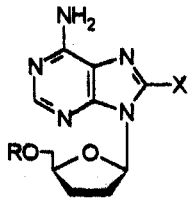
3a; R = H
b; R = TBDMS



4a; R = H
b; R = Ac



5a; $R^1 = NH_2$, $R^2 = R^3 = H$;
b; $R^1 = NH_2$, R^2 = TBDMS, $R^3 = H$;
c; $R^1 = NH_2$, R^2 = TBDMS, R^3 = IThC;
d; $R^1 = R^2 = R^3 = H$;
e; $R^1 = R^3 = H$, R^2 = DMTr;
f; R^1 = H, R^2 = DMTr, R^3 = IThC;



6a; R = H, X = H;
b; R = H, X = Br;
c; R = Ac; X = Br;

DMTr = 4,4'-dimethoxytrityl;
IThC = (imidazol-1-yl)thiocarbonyl;
TBDMS = *tert*-butyldimethylsilyl;

glycosidic bond of the latter in the acidic pH range we carried out the experiments in concentrated acetate or phosphate buffers. Compound **1a**, soluble in buffer underwent solvolysis and provided only traces of the desired product **3a**. On the contrary, poor solubility of 5'-O-*tert*-butyldimethylsilyl-2',3'-dideoxyguanosine **1b** and extremely poor solubility of its 8-bromo derivative (**3b**) allowed us to slow down the solvolytic cleavage and obtain **3b** in 55% yield. The optimal conditions for this transformation were the following: 1 M acetate buffer pH 4.5, 2.18 molar excess of bromine as saturated bromine water, 1.5 h at 20°C. When the reaction was performed in water for 0.5 h, it furnished a 36% yield of **3b**. Desilylation with tetra-n-butylammonium fluoride in pyridine resulted in pure **3a** in 81% yield.

A series of 8-substituted 2',3'-dideoxyinosine derivatives e.g. 8-amino-, 8-hydroxy[17] and 8-mercapto[18] have been conveniently prepared from their adenosine counterparts by hydrolytic deamination with adenosine deaminase. This approach could not be applied for preparation of 8-bromo-2',3'-dideoxyinosine **4a** from already described 8-bromo-2',3'-dideoxyadenosine (**6b**)[19] because 8-bromoadenosine has been found highly resistant to this enzyme.[19]

Encouraged by the successful bromination of **1a** in the form of poorly soluble **1b** in the heterogeneous medium, we attempted the same approach in the case of **2a**. We first used 5'-O-*tert*-butyldimethylsilyl-2',3'-dideoxyinosine (**2c**) suspended in acetate buffer at pH 5. Unlike **1b**, hypoxanthine - bearing substrate **2c** did not react with the excess of bromine even when warmed up to 60°C. Unprotected **2a** was also poorly soluble in acetate buffer. Direct bromination of that starting material in suspension occurred, but proceeded very slowly. Prolongation of the reaction time resulted in almost complete decomposition of the already preformed product **4a** to 8-bromo-hypoxanthine while most of **2a** remained unreacted. By careful investigations the following optimal conditions were settled: 1.5 M acetate buffer pH 4.65, 1.3 molar excess of bromine, 7 h at 20°C. Yield of the product **4a** initially estimated by TLC was approximately 20%. After tedious work-up procedure which was necessary to remove 2,3-dideoxyribose, the yield of pure 8-bromo derivative **4a** was 12%; 2,3-dideoxyribose and **4a** showed similar chromatographic mobility.

An attempt to accomplish the bromination of **2a** in solution in acetate buffer/methanol medium following the conditions described for bromination of 2',3'-

dideoxyadenosine (**6a**)[19] was unsuccessful. In this case we observed fast acid catalysed hydrolysis of preformed **4a** while unreacted **2a** predominated in the reaction mixture.

We also applied a method patterned after bromination of 2',3',5'-tri-O-acetylinosine with N-bromoacetamide in chloroform.[20] 5'-O-Acetyl-2',3'-dideoxyinosine[21] (**2d**) underwent partial substitution with bromine when refluxed in chloroform with excess of N-bromosuccinimide. 5'-O-Acetyl-8-bromo-2',3'-dideoxyinosine (**4b**) was isolated in 9% yield together with degradation products 8-bromohypoxanthine and hypoxanthine. Numerous further experiments in which various reaction conditions were used (ethylene chloride as another solvent, various reaction times, factors favouring either ionic or radical course of reaction) did not improve the original result.

In view of the recent report on satisfactory chemical deamination of adenosine 5'-thioether derivatives to the corresponding inosine and nebularine derivatives[22] it seemed of interest to use this method for the preparation of the desired **4a**. For this purpose, 8-bromo-2',3'-dideoxyadenosine[19] **6b** was acetylated with acetic anhydride in pyridine to give 5'-O-acetyl-8-bromo-2',3'-dideoxyadenosine (**6c**). Compound **6c** reacted readily with n-pentyl nitrite in refluxing tetrahydrofuran. According to TLC the starting material was transformed into two products, which after the work-up gave two other non-nucleosidic compounds as shown by ^1H and ^{13}C NMR analysis.

Studies on the biological properties of 8-bromo derivatives **3a**, **3b** and **4a** will be published elsewhere.

^1H and ^{13}C NMR characteristics

It has been shown for a number of purine 9-(β-D-ribofuranosides), 9-(β-D-2'-deoxyribofuranosides) and their 5'-monophosphates that substitution of their 8-position with various bulky substituents causes characteristic chemical shift changes in the carbohydrate portion of their NMR spectra. The signal of H-2' is shifted to higher frequencies and that of C-2' to lower frequencies.[3,4,23,24] These shifts have been assumed to be due to the syn conformation of 8-substituted purine nucleosides placing the lone pair of electrons of the base N-3 close to the sugar C-2'–H-2' bond.[25]

Along this line Nair and Young established an empirical rule correlating syn-anti conformation of purine ribofuranosides in solution with ^{13}C NMR chemical shift difference between C-2' and C-3'. According to it the unprotected compounds in the anti conformation exhibit Δ(C-2'−C-3') greater than 2.8 ppm and those in syn conformation Δ(C-2'−C-3') smaller than 0.5 ppm.[26]

In Table 1 and Table 2 we summarized the ^1H and ^{13}C NMR data of parent purine 2',3'-dideoxynucleosides **1a, 1b, 2a, 2d** and **6a** and their corresponding 8-bromo derivatives **3a, 3b, 4a, 4b** and **6b** in DMSO-d_6.

Upon substitution of the 8 position we observed characteristic chemical shift changes not only for H-2' and C-2' but also for H-3' and C-3' signals. The changes concerning H-2' and H-3' have not been described so far. In the previously published ^1H NMR spectra of a series of other 8-substituted purine 2',3'-dideoxynucleosides (e.g. 8-methylthio- and 8-amino-2',3'-dideoxyadenosine) in DMSO-d_6[17] or in the spectrum of 8-bromo-2',3'-dideoxyadenosine **6b** in $CDCl_3$,[19] the signals of H-2' and H-3' have been reported to appear as a four proton multiplet.[17,19]

In the case of 8-bromoguanine- and 8-bromoadenine-2',3'-dideoxynucleosides (**3a, 3b** and **6b** respectively), 2' and 3' protons appeared as a conspicuous pattern of three multiplets integrating for 1H, 2H and 1H, which on the basis of 2D experiment were ascribed, in order of lowering frequency, to H-2', H-2"+H-3' and H-3". For the corresponding 8-bromohypoxanthine derivatives **4a** and **4b**, the signals of the four studied protons were completely separated. Chemical shift changes towards higher frequencies of the H-2' signals in compounds **3a, 3b, 4a, 4b** and **6b**, being in the range 0.33-0.5 ppm, were similar to those described earlier for the corresponding 9-(β-D-ribofuranosyl) and 9-(β-D-2'-deoxyribofuranosyl) derivatives[3,23,24] as diagnostic for anti → syn conformational changes. New diagnostic features emerged, specific for 2',3'-dideoxynucleosides namely the shifts to higher frequencies of H-3' (0.22-0.35 ppm) and of C-3' (1.18-1.56 ppm) signals. These effects had been reported as very weak in the corresponding 8-bromo derivatives of the 9-(β-D-ribofuranosyl) series (not greater than 0.1[23] and 0.25[4] ppm, respectively).

Chemical shift changes to lower frequencies of C-2' signal were in the range 2.88-3.49 ppm, analogous to those reported for the ribo series.[4]

TABLE 1. ^{1}H NMR Data of Purine 2',3'-Dideoxynucleosides and their 8-Bromoderivatives. Chemical shifts δ_H (ppm) in DMSO-d_6-TMS and Coupling Constants (Hz).

Compd	H-2	H-8	H-1' ($J_{1',2'}$;$J_{1',2''}$)	H-2'	H-2"	H-3'	H-3"
1a[a]	-	7.96 s,1	6.00 dd,1 (6.6;3.6)	[c]2.30 m,2		2.01 m,2	
3a[b]	-	-	6.03 dd,1 (7.8;4.7)	2.70 m,1	2.30 m,2		2.02 m,1
1b	-	7.86 s,1	5.97 dd,1 (6.7;3.2)	[c]2.30 m,2		1.99 m,2	
3b	-	-	6.03 dd,1 (7.5;3.9)	2.80 m,1	2.34 m,2		2.01 m,1
2a[a]	8.34 s,1	8.06 s,1	6.20 dd,1 (6.7;3.3)	[c,d]2.40 m,2		2.03 m,2	
4a[b]	8.09 s,1	-	6.16 dd,1 (7.8;4.7)	2.78 m,1	[d]2.45	2.25 m,1	2.06 m,1
2d	8.23 s,1	8.06 s,1	6.22 dd,1 (6.5;4.1)	[d]2.47		2.11 m,2	
4b	8.09 s,1	-	6.21 dd,1 (7.6;4.0)	2.80 m,1	[c,d]2.43		2.12 m,1
6a[a]	8.36 s,1	8.15 s,1	6.23 [e]pt,1	2.41 m,2		2.04 m,2	
6b[a,b]	8.13 s,1	-	6.17 m,1	2.88 m,1	2.35 m,2		2.09 m,1

[a] ^{1}H NMR data already published: **1a**, **6a**[9]; **2a**[11]; **6b**($CDCl_3$)[18]. [b] 2D Spectrum measured. [c] Center of two partially overlapping multiplets. [d] Partially overlapping DMSO-d_5. [e] pt: pseudotriplet.

(continued)

TABLE 1. continued.

H-4'	H-5' [J_{gem};$J_{5'(5''),4'}$]	H-5"	5'-OH	N-1-H	NH_2	5'-OR
4.06 m,1	[c]3.55 m,2		4.96 t,1,ex [6.0]	10.64 brs,1 ex	6.48 brs,2 ex	-
4.03 m,1	3.52 t,2 [5.6]		4.79 t,1,ex [5.9]	10.80 brs,1 ex	6.50 brs,2 ex	-
4.06 m,1	3.76 dd,1 [11.2;3.6]	3.65 dd,1 [11.2;4.5]	-	10.59 brs,1 ex	6.47 brs,2 ex	0.84 s,9,$(CH_3)_3C$ 0.01 s,6,$(CH_3)_2Si$
4.04 m,1	3.68 m,2		-	10.77 brs,1 ex	6.50 brs,2 ex	0.80 s,9,$(CH_3)_3C$ 0.08,s,3 0.05,s,3 $(CH_3)_2Si$
4.11 m,1	3.63 dd,1 [11.8;3.5]	3.51 dd,1 [11.8;4.3]	4.98 brs,1 ex	12.35 brs,1 ex	-	-
4.07 m,1	3.49 m,2		4.79 m,1 ex	12.53 brs,1 ex	-	-
4.30 m,1	4.20 dd,1 [12.0;3.4]	4.13 dd,1 [12.0;6.2]	-	12.41 brs,1 ex	-	1.98 s,3 $COCH_3$
4.28 m,1	4.09 m,2		-	12.56 brs,1 ex	-	1.91 s,3 $COCH_3$
4.12 m,1	3.62 m,1	3.53 m,1	5.08 t,1,ex [5.6]	-	7.28 brs,2 ex	-
4.10 m,1	3.55 dd,1 [11.5;4.1]	3.44 dd,1 [11.5;5.4]	5.06 brs,1 ex	-	7.46 brs,2 ex	-

TABLE 2. ^{13}C NMR Data of 2',3'-Dideoxynucleosides and their 8-Bromoderivatives. Chemical shifts δ_C (ppm) in DMSO-d_6-TMS.

Compd	C-2	C-4	C-5	C-6	C-8	C-1'	C-2'	C-3'	Δ(C-2'-C-3')	C-4'	C-5'
1a	153.47 S	150.47 S,m	116.63 Sd	156.69 S	135.05 Dd	83.54 Dm	31.80 Tm	25.53 Tm	6.27	81.53 Dm	62.73 Td
3a	153.32 S	151.81 Sd	117.37 S	155.49 S	120.43 Sd	85.80 Dm	28.55 Tm	27.09 Tm	1.46	81.42 D	63.74 Tm
1b[a]	153.56 S	150.54 Sd	116.68 S	156.72 S	134.76 Dd	83.47 Dm	31.79 T	25.35 T	6.44	81.12 Dm	64.44 T
3b[b]	153.23 S	151.74 Sd	117.25 S	155.45 S	120.58 Sd	85.82 Dm	28.30 Tm	26.81 Tm	1.49	81.16 Dm	65.16 Tm
2a	145.68 D	147.66 Sdd	124.38 Sd	156.68 Sd	138.25 Dd	84.52 D	32.21 T	25.52 T	6.69	82.11 D	62.71 T
4a	145.79 D	148.86 Sdd	125.03 S	155.17 Sd	125.24 Sd	86.70 D	28.90 T	26.87 T	2.03	81.67 D	63.43 T
6a	152.29 D	148.71 Sddd	119.00 Sdt	155.90 Sd	138.92 Dd	84.32 D	31.63 T	25.60 T	6.03	81.57 D	62.85 T
6b	152.31 D	149.80 Sdd	119.46 St	154.85 Sd	126.46 Sd	86.82 D	28.75 T	26.78 T	1.97	81.38 D	63.64 T

[a] **1b** δ_c (ppm) of TBDMS carbon atoms: 25.81, 18.02, -5.47.
[b] **3b** δ_c (ppm) of TBDMS carbon atoms: 25.67, 17.90, -5.41, -5.53.

We analyzed the ^{13}C NMR data shown in Table 2 also from the viewpoint of the possible extension of the above mentioned Δ(C-2'−C-3') rule to the dideoxy series. For parent dideoxynucleosides **1a, 1b, 2a** and **6a** we found chemical shift differences between C-2' and C-3' in the range of 6.03-6.69 ppm. It seems that Δ(C-2'−C-3') value of approx. 6 ppm may be safely considered indicative of the preferred anti conformation around the glycosidic bond of the 2',3'-dideoxynucleoside. It has been found previously by a semiquantitative 1D ^1H NOE differential method that 2',3'-dideoxyguanosine **1a**, 2',3'-dideoxyadenosine **6a** and a derivative of 7-deazapurine, 4-chloro-7-(2,3-dideoxy-β-D-glyceropentofuranosyl)-7*H*-pyrrolo[2,3-d]-pyrimidine, exhibit preferentially anti conformation.[27] The Δ(C-2'−C-3') value for the last compound calculated from literature data[28] is also 6.1 ppm. Furthermore, **6a** is known from X-ray crystallographic data to exist in the anti conformation in the solid state.[11]

On the other hand, for 8-bromo counterparts **3a, 3b, 4a** and **6b** the chemical shift differences between C-2' and C-3' are found in the range of 1.46-2.03 ppm and reflect dominating syn conformation. Δ(C-2'−C-3') in the similar range, 1.9−2.2 ppm, can be calculated from ^{13}C NMR data reported[17] for 8-methylthio-, 8-methoxy- and 8-benzyloxy-2',3'-dideoxyadenosine; for the less bulky 8-amino- substituent that value is 3 ppm.

From the above it may be concluded that similarly to ribo series, the glycosidic bond conformation of purine 2',3'-dideoxynucleosides in solution expressed in terms of preferred anti or syn conformation can be conveniently qualitatively determined by the magnitude of the ^{13}C NMR chemical shift difference between C-2' and C-3'.

EXPERIMENTAL SECTION

Melting points were determined in open capillaries on Laboratory Devices MEL-TEMP II capillary micromelting point apparatus and are uncorrected. UV spectra were measured in water or methanol on Beckman DU-65 Spectrophotometer. High resolution mass spectrum of **4a**, measured in Mass Spectrometry Laboratory of the Institute of Organic Chemistry of the Polish Academy of Sciences in Warsaw, was obtained by LSIMS method on AMD-604 mass spectrometer - the sample in m-nitrobenzyl alcohol matrix (NBA) was bombarded with cesium ions of energy 10 kV.

High resolution data were obtained by Peak Matching technique; as a reference peak [2 NBA + H]$^+$ ion was used.

Elemental analyses were performed by Microanalytical Laboratories of the aforementioned Institute in Warsaw.

Analytical thin-layer (TLC) and preparative-layer chromatography (PLC) were conducted on Merck precoated silica gel F^{254} type 60 plates of layer thickness 0.25 and 0.5 mm respectively. For preparative short-column chromatography Merck TLC silica gel type 60 H and silanized silica gel type 60 H were used. All solvents used were of the reagent grade. Dried dioxane and tetrahydrofuran purified from peroxides, were refluxed over calcium hydride and distilled prior to use. n-Pentyl nitrite[29] was dried over sodium sulphate, distilled and stored at 10°C over 4- Å molecular sieves. ^{1}H and ^{13}C spectral measurements - ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on Unity 300 Varian spectrometer operating at 299.95 MHz and 75.43 MHz respectively. 2D ^{1}H NMR COSY spectra were measured in DMSO-d_6 using mode Absolute Value with a resolution of 6 Hz in both directions (Me_4Si as internal reference, chemical shifts expressed as δ values).

5'-O-(*tert*-Butyldimethylsilyl)-2',3'-dideoxyguanosine (**1b**) To a stirred at 5°C suspension of dried 2'-deoxyguanosine (0.81 g, 3 mmol) and imidazole (0.82 g, 12 mmol) in DMF (24 ml) was added *tert*-butyldimethylsilyl chloride (0.90 g, 6 mmol). The reaction mixture was stirred at 5°C for 15 min and then quenched with methanol (0.5 ml). The solvent was removed under vacuum and the residue was stirred with cold water (45 ml). The white precipitate was collected, washed with water and dried, redissolved in methylene chloride (45 ml) and stirred for 1 h to wash out traces of disubstituted product. 5'-O-(*tert*-butyldimethylsilyl)-2'-deoxyguanosine (**5b**) was filtered off and dried to yield 0.93 g of white solid (82%, m.p. >221°C dec.; lit.[12] m.p. >230°C dec.). ^{1}H NMR (DMSO-d_6) δ: 0.03 (6H, s, Me_2Si), 0.86 (9H, s, Me_3CSi), 2.36-2.52 (m, overlaps with DMSO-d_5, H-2'), 3.73-3.81 (3H, m, H-4', H-5'), 4.36 (1H, m, H-3'), 5.09 (1H, br s, exchangeable, 3'-OH), 6.12 (1H, t, H-1'), 6.57 (2H, br s, exchangeable, NH_2), 7.83 (1H, s, H-8), 10.90 (1H, br s, exchangeable, NH).

To a solution of 5'-O-(*tert*-butyldimethylsilyl)-2'-deoxyguanosine **5b** (0.54 g, 1.42 mmol) in DMF (2 ml), 1,1'-thiocarbonyldiimidazole (0.50 g, 2.84 mmol) was added. It was set aside for 20 h, then the volatiles were removed. The residue treated with

ethyl acetate (3 ml) yielded crystalline 5'-O-(*tert*-butyldimethylsilyl)-3'-O-(imidazol-1-yl)thiocarbonyl-2'-deoxyguanosine (**5c**) 0.53 g (75%, m.p. 208-210°C dec.).

^{1}H NMR (DMSO-d_6) δ: 0.06 (6H, s, Me_2Si), 0.86 (9H, s, Me_3CSi), 2.82-3.90 (4H, complex, H-2', H-5'), 4.44 (1H, m, H-4'), 5.99 (1H, m, H-3') 6.28 (1H, dd, J: 6.5, 3.7 Hz, H-1'), 6.51 (2H, br s, exchangeable, NH_2), 7.12 (1H, m, imidazole), 7.88-7.90 (2H, m, imidazole and H-8), 8.57 (1H, s, imidazole), 10.67 (1H, br s, exchangeable, NH).

To a suspension of 5'-O-(*tert*-butyldimethylsilyl)-3'-O-(imidazol-1-yl)thiocarbonyl-2'-deoxyguanosine (0.49 g, 1 mmol) in anhydrous dioxane (20 ml) at reflux a solution of tri-n-butyltin hydride (0.627 g, 2.15 mmol) and azobisisobutyronitrile (0.053 g, 0.32 mmol) in dioxane (7 ml) was added dropwise over 20 min. The mixture was refluxed for 1 h, cooled to 10°C. **1b** precipitated, it was carefully washed with dioxane, then with n-hexane, dried to yield 0.33 g (91%) of material homogeneous by TLC: m.p. >188°C dec. did not raise after recrystallization from butan-2-one. NMR data of **1b** given in Tables 1, 2.

2',3'-Dideoxyguanosine (**1a**). To a solution of **1b** (0.236 g, 0.65 mmol) in anhydrous pyridine (8 ml) a 1 M solution of tetra-n-butylammonium fluoride in THF (1.3 ml, 1.3 mmol) was added. The mixture was set aside overnight. The volatiles were removed under vacuum and the residue treated with water (4 ml) and filtered through Celite pad. The filtrate was applied to a column packed with silanized silica gel (60 x 50 mm) which was eluted first with water then with aqueous 10% acetone. Fractions containing **1a** were pooled and evaporated to dryness. Crystallization of **1a** from hot methanol afforded 0.125 g of white solid (77%, m.p. >250°C, the same as reported[11]).

5'-O-(4,4'-Dimethoxytrityl)-2'-deoxyinosine (**5e**). 2'-Deoxyinosine (**5d**) (0.40 g, 1.59 mmol) in anhydrous pyridine (40 ml) was treated with 4,4'-dimethoxytrityl chloride (0.66 g, 2.0 mmol) according to the described procedure.[15] Product **5e** was purified by column chromatography on silica gel with 5-20% methanol in chloroform (with 0.2% Et_3N). It afforded 0.71 g (81%) of white foam. ^{1}H NMR (DMSO-d_6) δ: 2.35 (1H, m, H-2"), 2.77 (1H, m, H-2'), 3.15 (2H, m, H-5'), 3.72 (6H, s, methoxy groups), 3.97 (1H, m, H-4'), 4.42 (1H, m, H-3'), 5.38 (1H, d, exchangeable, 3'-OH),

6.34 (1H, t, H-1'), 6.68 (4H, m, dimethoxytrityl), 7.18-7.31 (9H, m, dimethoxytrityl), 7.99 (1H, s, H-8), 8.18 (1H, m, H-2) 10.36 (1H, s, exchangeable, NH).

5'-O-(4,4'-Dimethoxytrityl)-2',3'-dideoxyinosine (**2b**).

a) A solution of **5e** (0.60 g, 1.1 mmol) in DMF (6 ml) was reacted with 1,1'-thiocarbonyldiimidazole (0.39 g, 2.2 mmol) to form 5'-O-(4,4'-dimethoxytrityl)-3'-O-(imidazol-1-yl)thiocarbonyl-2'-deoxyinosine **5f** in 20 h. The volatiles were removed under vacuum and the residue was directly used in step b).

A portion of **5f** was purified by PLC with 5% MeOH in $CHCl_3$ (with 0.2% Et_3N) and characterized by ^1H NMR (DMSO-d_6) δ: 2.90 (1H, m, H-2"), 3.35 (3H, m, H-2', H-5'), 3.72 (6H, s, methoxy groups), 4.58 (1H, m, H-4'), 6.07 (1H, m, H-3'), 6.53 (1H, dd, J: 7.3, 6.2 Hz, H-1'), 6.82 (4H, m, dimethoxytrityl), 7.13-7.38 (10H, m, 9H dimethoxytrityl, 1H imidazole), 7.91-7.96 (2H, m, imidazole), 8.25 (1H, s, H-8), 8.61 (1H, s, H-2), 12.45 (1H, br s, exchangeable, NH).

b) To a solution of crude **5f** (ca 1.1 mmol) in anhydrous dioxane (50 ml) at reflux, a solution of tri-n-butyltin hydride (1.716 g, 5.85 mmol) and azobisisobutyronitrile (0.143 g, 0.087 mmol) in dioxane (10 ml) was added dropwise for 30 min. After the reaction was completed (1 h) the volatiles were removed under reduced pressure. The residue was partitioned between n-hexane and acetronitrile. The acetonitrile layer was washed with n-hexane, concentrated under vacuum and purified by chromatography over silica gel eluting with 1-7% MeOH in chloroform (with 0.2% Et_3N) to yield 0.496 g (84%) of **2b** as off-white foam.

Chromatography over silica gel eluting first with 0.2% Et_3N in n-hexane (v/v) then with MeOH:Et_3N:methylene chloride 2.8:0.2:97 (v/v/v) yielded an analytical sample of **2b** as white foam: UV [MeOH] λ_{max} 236 {25500} sh 273 {6670} 282 {4530}. ^1H NMR (DMSO-d_6) δ: 2.07 (2H, m, H-3'), 2.50 (m, overlaps with DMSO-d_5, H-2'), 3.09 (2H, m, H-5'), 3.72 (6H, s, methoxy groups), 4.26 (1H, m, H-4'), 6.26 (1H, dd, J: 5.7, 4.0 Hz, H-1'), 6.81 (4H, m, dimethoxytrityl), 7.16-7.32 (9H, m, dimethoxytrityl), 8.06 (1H, s, H-8), 8.19 (1H, s, H-2), 12.38 (1H, br s, exchangeable, NH).

Anal. Calcd for $C_{31}H_{30}N_4O_5$ + 1/2 H_2O: C, 67.99; H, 5.71; N, 10.23; Found C, 67.98; H, 5.64; N, 10.17.

2',3'-Dideoxyinosine (2a). **2b** (0.810 g, 1.5 mmol) cooled in an ice bath was treated with 80% aqueous acetic acid (20 ml) at 0°C. The mixture was stirred at 0°C until TLC revealed the absence of starting material (1.5 h). It was then carefully neutralized dropwise with conc. aqueous ammonia at 0°C, finally pH was adjusted to 9.5 and 60 ml of cold ethanol was added. The volatiles were removed under vacuum and the residue was partitioned between methylene chloride (30 ml) and water (30 ml). The aqueous layer was concentrated to ca 5 ml and applied on a column packed with silanized silica gel (60 x 50 mm). The column was washed first with water (200 ml) to remove salts, then with 10-25% aqueous acetone. Fractions containing pure **2a** were pooled and evaporated to yield a white solid, later crystallized from methanol to form 0.227 g (65%) of **2a** as white crystals: m.p. soft. 180-185°C, dec. 197°C (lit[11] [EtOH-H_2O] soft. 184-186°C, does not melt up to 300°C). ^1H and ^{13}C NMR data presented in Tables 1, 2.

5'-O-(*tert*-Butyldimethylsilyl)-2',3'-dideoxyinosine (2c). **2a** (0.047 g, 0.2 mmol) was coevaporated with anhydrous pyridine, then suspended in pyridine (2 ml). *tert*-Butyldimethylsilyl chloride (0.054 g, 0.36 mmol) was added and the resulting mixture was stirred overnight. It was treated with sodium bicarbonate sat. solution (10 ml) and extracted with chloroform (3 x 5 ml). Organic layers were combined, dried with sodium sulphate and evaporated under vacuum. The residue was triturated with anhydrous n-hexane (10 ml), **2c** was filtered off, washed with n-hexane and dried over P_2O_5 to yield 0.066 g (89%): m.p. 168-170°C. ^1H NMR (DMSO-d_6) δ: 0.01 (6H, s, Me_2Si), 0.85 (9H, s, Me_3CSi), 2.04 (2H, m, H-3'), 2.44 (H-2', overlaps with DMSO-d_5), 3.68 (1H, dd, J: 11.4, 3.8 Hz, H-5"), 3.82 (1H, dd, J: 11.4, 3.8 Hz, H-5'), 4.15 (1H, m, H-4'), 6.21 (1H, dd, J: 6.8, 3.1 Hz, H-1'), 8.04 (1H, s, H-8), 8.26 (1H, s, H-2), 12.33 (1H, br s, exchangeable, N-1 H).

5'-O-Acetyl-2',3'-dideoxyinosine (2d).[21] **2a** (0.121 g, 0.51 mmol) was co-evaporated with anhydrous pyridine suspended in 2 ml of pyridine and treated with acetic anhydride (0.068 g, 0.66 mmol). The mixture was stirred overnight then coevaporated with toluene (3 x 10 ml) under vacuum. The sole product **2d** was recrystallized from hot methanol (10 ml) to give 0.121 g (85%) of white crystals: m.p. 165-167°C, UV[H_2O] λ_{max} 249 {7900}. ^1H NMR data given in Table 1.

8-Bromo-5'-O-(*tert*-butyldimethylsilyl)-2',3'-dideoxyguanosine (**3b**). To a stirred at 20°C suspension of **1b** (0.200 g, 0.55 mmol) in acetate buffer (6 ml, 1 M, pH 4.5) bromine water [0.060 ml Br_2 (0.19 g, 1.2 mmol) in 5.3 ml H_2O] was added dropwise in 2 portions: 2.5 ml within 30 min, pause of 20 min, the other portion in 30 min. The reaction was continued for 15 min more, then refrigerated for 30 min. Precipitated product **3b** was filtered off, carefully washed with acetate buffer (20 ml, 1 M, pH 5) then with cold water. It afforded 0.196 g of crude material. **3b** was purified by column chromatography (40 x 60 mm) on silica gel with chloroform/ /ethanol/triethylamine (90:9:1 v/v/v) as the eluent. Fractions containing pure **3b** were combined and concentrated under vacuum to ca 4 ml volume. Upon cooling, it slowly crystallized to give analytically pure **3b** as white, shiny flakes (0.058 g, 24%, m.p. 197°C, UV [MeOH] λ_{max} 262 {14260}). The filtrate afforded 0.075 g (31%) of **3b**. ^1H and ^{13}C NMR data presented in Tables 1, 2.

Anal. Calcd for $C_{16}H_{26}BrN_5O_3Si$: C, 43.24; H, 5.90; N, 15.76; Br, 17.98. Found C, 43.19; H, 6.27; N, 15.84; Br, 17.70.

8-Bromo-2',3'-dideoxyguanosine (**3a**). A solution of **3b** (0.127 g, 0.28 mmol) in anhydrous pyridine (5 ml) was treated with 1 M solution of tetra-n-butylammonium fluoride in THF (0.9 ml) and set aside overnight. It was coevaporated with toluene under vacuum below 20°C to an oily residue. The product **3a** was isolated by chromatography on silica gel column (40 x 60 mm) with 5-10% methanol in chloroform (with 0.2% Et_3N). Fractions containing pure **3a** were combined and evaporated below 20°C to the oily residue. It was dissolved in water (5 ml) and filtered. The filtrate was concentrated to ca 1.5 ml and refrigerated overnight. Resulting white crystals were filtered off, washed with icy water and dried to yield 0.056 g of analytically pure **3a** (56%, m.p. 195°C dec., UV [MeOH] λ_{max} 261 {15300}). The filtrate yielded additional 0.023 g (25%) of **3a** as off-white solid. ^1H and ^{13}C NMR data given in Tables 1, 2.

Anal. Calcd for $C_{10}H_{12}BrN_5O_3$ + 1/2 H_2O: C, 35.41; H, 3.86; N, 20.65; Br, 23.56. Found C, 35.67; H, 3.90; N, 20.45; Br, 23.27.

8-Bromo-2',3'-dideoxyinosine (**4a**). To a stirred solution of **2a** (0.047 g, 0.2 mmol) in 1.5 M acetate buffer pH 4.65 (1 ml), bromine (0.041 g, 0.26 mmol) was added at 20°C. The solution turned heterogeneous and cleared again after 6 h of intensive

stirring. The reaction was continued for 1 more hour then it was stopped by cooling to 0°C and pH of the solution was carefully adjusted to 7 with cold 1 M phosphate buffer (pH 7.2, 15 ml). Unreacted bromine was removed under vacuum below 10°C. The residual mixture was applied to a column packed with silanized silica gel (40 x 50 mm). The column was first washed with water (200 ml) to remove the salts, then with a gradient of acetone in water (1-15%). Fractions ca 10 ml were collected: #7-11 contained **2a** (recovered 0.031 g), #14-15 contained **4a** (0.015 g contaminated with UV not-absorbing material). Finally, **4a** was purified by TLC: it was applied on 2 plates (0.25 mm each). The separation was conducted with chloroform/ ethanol/ water 40:10:1 (v/v/v) as a solvent system (it contained 0.2% Et_3N) at 4°C. The bands were cut off and the product **4a** was eluted with the same solvent system, filtered through silica gel 10 mm pad. The filtrate was evaporated under vacuum below 15°C to yield 0.007 g (12%) of **4a** as white solid: m.p. soft. 126°C, dec. 177°C. UV [H_2O] λ_{max} 254 {15200}.

4a: HR MS sample mass 315.0089, calcd for $C_{10}H_{12}BrN_4O_3$ $(M+H)^+$: 315.0093. ^1H and ^{13}C NMR data presented in Tables 1, 2.

5'-O-Acetyl-8-bromo-2',3'-dideoxyinosine (**4b**). To a protected from moisture solution of **2d** (0.042 g, 0.15 mmol; dried in vacuo: 0.01 Torr, 30°C, 3 h) in anhydrous chloroform (3 ml) at reflux N-bromosuccinimide was added (0.134 g, 0.75 mmol). The resulting suspension was heated at reflux for 8 h. After that time, the reaction mixture was cooled to remove the excess of NBS. The solution was decanted and applied on 2 PLC plates. The separation was conducted in darkness at 4°C with chloroform/methanol 95:5 (v/v) as a solvent system. Bands corresponding to **4b** were cut off and the product was eluted from silica gel with the same solvent system. It yielded 0.005 g of **4b** (9%): UV pH λ_{max} 235 {13090}, pH 11 λ_{max} 258 {12200}. ^1H NMR data presented in Table 1.

Other experimental conditions were investigated. When a mixture of **2d** and NBS (5 eq) in chloroform was refluxed with benzoyl peroxide in argon atmosphere for 6 h, only minute amounts of **4b** were observed by TLC. When **2d** and NBS were heated in ethylene chloride up to 60°C in the presence of $ZnCl_2$ for 4-8 h traces of **4b** were observed followed by depurination of the starting material.

8-Bromo-2',3'-dideoxyadenosine[19] (**6b**). A solution of 2',3'-dideoxyadenosine (0.120 g, 0.5 mmol) in methanol (5 ml) - 1 M acetate buffer pH 4.65 (3 ml) was treated with bromine (0.175 g, 1.1 mmol) and kept for 3 h at 20°C. Bromine and methanol were removed in vacuo. The residual solution was treated with 1 M phosphate buffer pH 7 (6 ml) and extracted with pyridine/methylene chloride (1:3, 4 x 7 ml). Organic layers were combined and evaporated, the residue was purified by chromatography on silica gel with 5% methanol in methylene chloride. It yielded 0.135 g (86%) of **6a**: m.p. soft. 159°C (lit.[19] m.p. soft. 164°C). ^1H and ^{13}C NMR data presented in Tables 1, 2.

5'-O-Acetyl-8-bromo-2',3'-dideoxyadenosine (**6c**). **6b** (0.133 g, 0.42 mmol) was dried by coevaporation with 2 successive volumes of anhydrous pyridine (5 ml) and dissolved in pyridine (2 ml). Acetic acid anhydride (0.086 g, 0.084 mmol) was added at 0°C. After the reaction was completed (12 h), it was cooled to 0°C and MeOH (0.5 ml) was added. The volatiles were removed under vacuum. The product **6c** was purified by chromatography on a silica gel column with 1-3% methanol in methylene chloride. It yielded 0.130 g (86%) of pure crystalline **6c**: m.p. 127-129°C, ^1H NMR (DMSO-d_6) δ: 1.85 (3H, s, CH_3), 2.09 (2H, m, H-3"), 2.45 (m, overlaps with DMSO-d_5, H-2", H-3'), 2.97 (1H, m, H-2'), 4.06 (1H, dd, J: 11.5, 6.4 Hz, H-5"), 4.21 (1H, dd, J: 11.5, 3.4 Hz, H-5'), 4.27 (1H, m, H-4'), 6.21 (1H, dd, J: 7.4, 3.8 Hz, H-1'), 7.42 (2H, br s, exchangeable, NH_2), 8.14 (1H, s, H-2).

Attempted conversion of **6c** to **4b** and 5'-O-acetyl-8-bromo-2',3'-dideoxy-nebularine with n-pentyl nitrite. To a stirred at reflux solution of **6c** (0.050 g, 0.14 mmol) in anhydrous tetrahydrofuran (4 ml), a solution of n-pentyl nitrite (0.40 ml, 2.8 mmol) in THF (4 ml) was added dropwise within 15 min. Gas evolution began and the reaction mixture turned yellow. Reflux was continued for 5 h until TLC revealed that the reaction was stopped. About 85% of **6c** underwent transformation - two UV absorbing products A and B were observed (Rfs: A 0.39, B 0.11 with 5% methanol in chloroform as TLC solvent system). The volatiles were removed under vacuum, below 20°C. An oily residue, cooled to 0°C was treated with ethanol (10 ml). The solvent was removed under vacuum. Neither product A nor B were found in the residue. It contained two other unidentified UV absorbing products C and D (Rfs: C 0.25, D 0.00 with 5% methanol in chloroform). C (UV [MeOH] λ_{max} 257, sh

262) and D (UV [MeOH] λ_{max} 260) were isolated by PLC with 10% methanol in chloroform containing traces of Et_3N.

^1H and ^{13}C NMR spectra of C and D measured in DMSO-d_6 confirmed that neither C nor D were nucleosides.

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