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STUDIES DIRECTED TOWARD SYNTHESIS OF GUANOSINE 8,5′-IMINO CYCLONUCLEOSIDES AND DERIVATIVES—I

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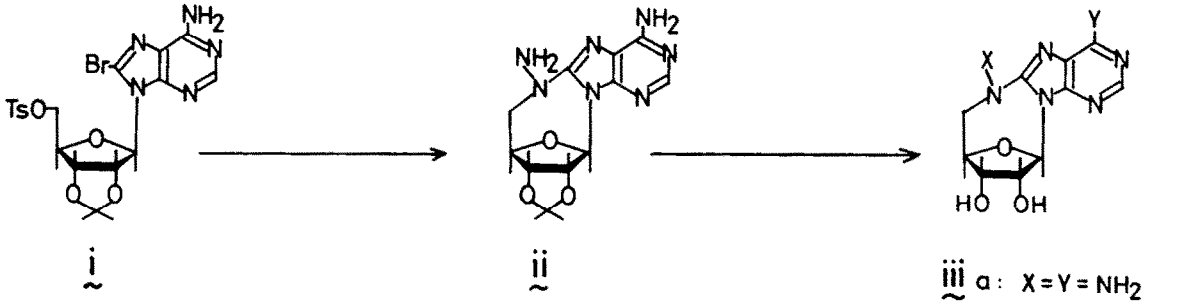
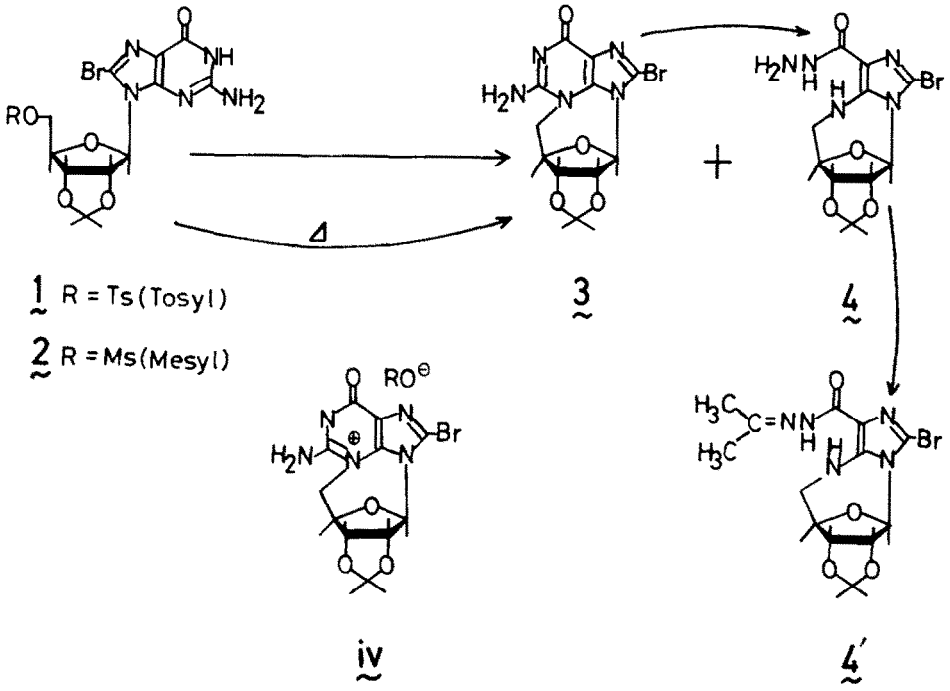
Abstract—8,5′-Aminimino bridging in the guanosine series using 5′-*O*-tosyl (**1**) and 5′-*O*-mesyl derivatives (**2**) of 2′,3′-*O*-isopropylidene-8-bromoguanosine (**5**) and hydrazine gave N^3,5′-cyclized product **3** and the N^3,5′-cyclonucleoside of 4-carboxyhydrazido-5-amino-2-bromoimidazole **4**. To exclude the N^3,5′-cyclization through ionization in the base moiety, a N^2-dimethylaminomethylidene-N^1-methoxymethylene derivative **7** was synthesized from **5** through the N^2-protected compound **6**. **7** was converted into the N^2-dimethylaminomethylidene-N^1-methoxymethylene-5′-*O*-mesyl analogue **8**, which with hydrazine yielded first the N^2-deprotected form of **8** (**9**). **8** or **9** with hydrazine under forcing conditions gave an 8,5′-aminimino-N^1-methoxymethyleneguanosine derivative **10**. Oxidation of **10** with sodium metaperiodate or sodium nitrite yielded 8,5′-imino-N^1-methoxymethyleneguanosine (**11a**) and 8,5′-imino-N^1-methoxymethylene-xanthosine derivative **11b**, respectively. **11a** was deprotected to 8,5′-imino-N^1-methoxymethyleneguanosine **12**.

We have recently described[1] the first synthesis of some purine 8,5′-imino cyclonucleosides (Scheme 1, **iiia–d**) through an 8,5′-aminimino bridged adenosine derivative **ii** as key intermediate and noted possible biological implications. In this case, 8,5′-aminimino bridging was achieved with a remarkably high yield by reacting hydrazine as a very strong nucleophile with 2′,3′-*O*-isopropylidene-5′-*O*-tosyl-8-bromoadenosine **i**. Since functionalization at C_2 in **ii** and/or **iii** for further transformations seemed to be intrinsically difficult, we chose to extend the same cyclization to guanosine derivatives, the bifunctional pyrimidine part of which was expected to allow further derivatization.

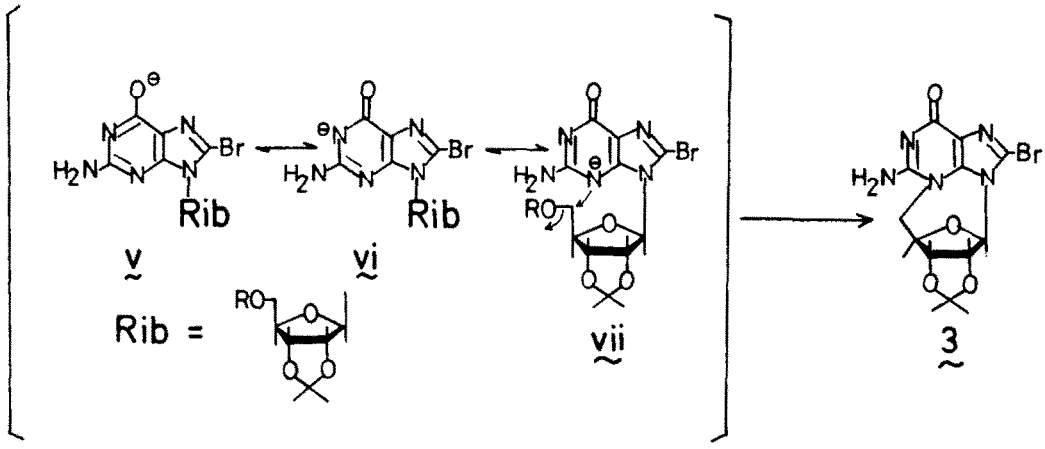
Although with 2′,3′-*O*-isopropylidene-5′-*O*-tosyl-8-bromoguanosine **1** as starting material a very facile intramolecular cyclization prior to its isolation is described,[2a] we succeeded in its synthesis in a sufficient yield by low temperature tosylation of 2′,3′-*O*-isopropylidene-8-bromoguanosine (**5**) (Scheme 4). While in the synthesis of **ii** from **i** as large as 44 fold excess hydrazine was used,[1] the first experiment with **1** was tried using a smaller quantity (20 fold excess) of hydrazine to exclude the hydrazinolysis of the lactam group in the base moiety. After 5.5 h reaction at room temperature, a high yield of a crystalline product was isolated. This compound contained bromine and indicated no IR absorption of a covalent sulfonate bond (1180 cm^{-1}) displayed by the starting material. In the ^{1}H-NMR spectrum, a large geminal coupling constant (14 Hz) for the 5′-methylene was observed and hence considered to be a cyclonucleoside. This compound was finally compared spectroscopically and shown to be identical with known 2′,3′-*O*-isopropylidene-5′,N^3-cyclo-8-bromoguanosine (**3**).[2a] Since we noted less polar minor products in this reaction and further the bulky 5′-*O*-tosyl group seemed to have accelerated the intramolecular cyclization,[2a] another longer-time reaction aiming at the isolation of the minor components was carried out, utilizing this time 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-bromoguanosine **2**.[2a] TLC analysis at an early stage of the reaction (after several hours) showed the same product distribution with the former reaction of **1**, the rapid formation of **3** being observed. After 67 h, a faster-running component was barely isolated and



Scheme 1.

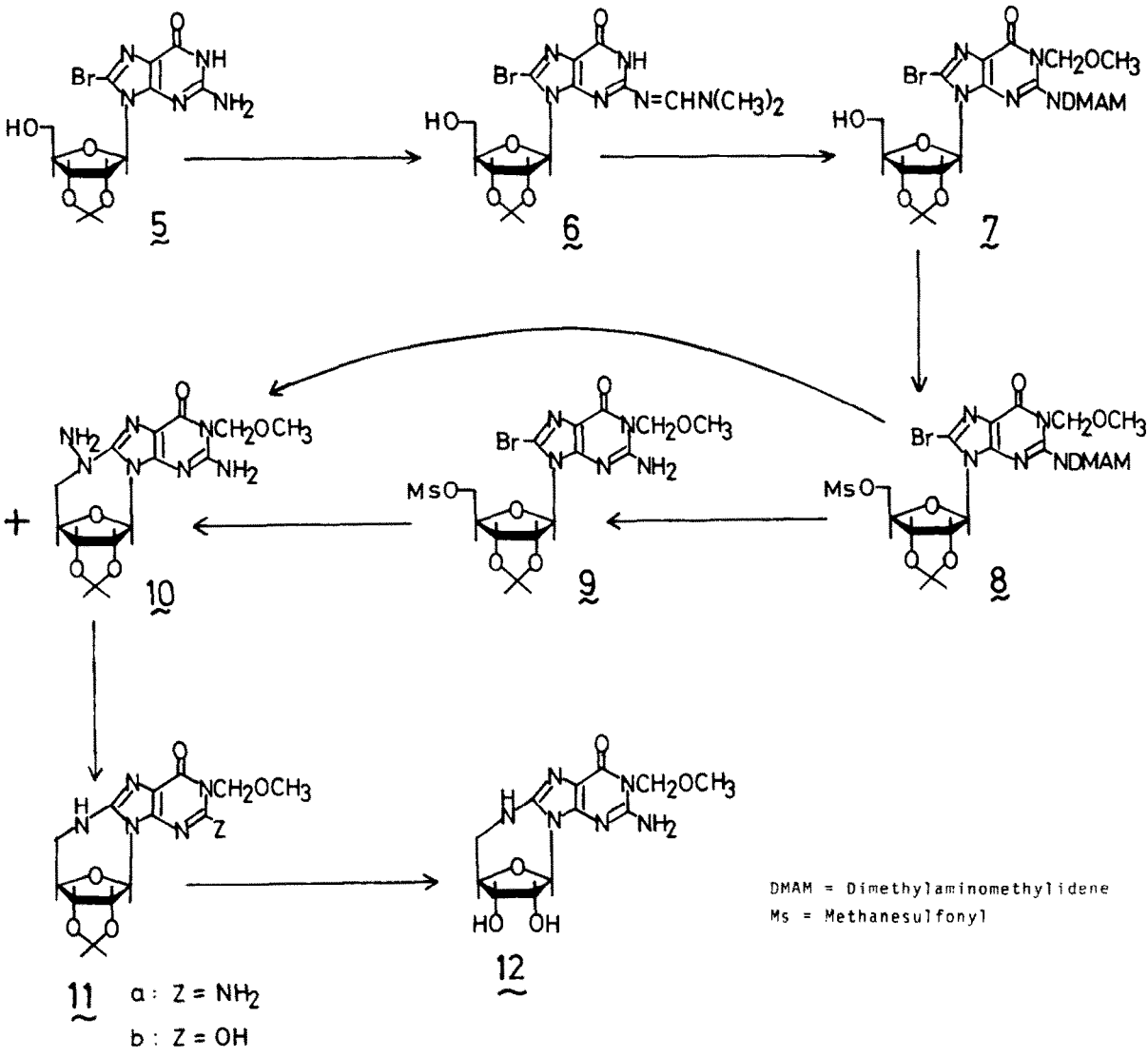


Scheme 2.



Scheme 3.

characterized as N^5,5'-anhydro-N^1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-4-carboxyhydrazido-5-amino-2-bromoimidazole **4**. **4** absorbs at 277 nm which coincides with the absorptions of some N^1-furanose derivatives[3,4] of 5-aminoimidazole-4-carboxamide. This compound appeared to be unstable probably due to air-oxidation and accordingly it was mostly isolated as an acetone hydrazone **4'**. This compound must have formed from **3** as judged by TLC during the reaction and also from a separate experiment using **3** and hydrazine. Thus, in these experiments 8,5'-cyclization was completely excluded in spite of the description that the ease of formation of a 3,5'-purine cyclonucleosides (quaternized forms) is related to the basicity of the heterocyclic system.[5-8] Since 8,5'-cyclo-2',3'-*O*-isopropylidene-8-mercaptoguanosine had been synthesized from **2** and thiourea in neutral media,[2a] it occurred to us that in our reactions anionic forms of **1** or **2** (Scheme 3, **v**, **vi** and/or **vii**) unusually accelerated the intramolecular cyclization. On the basis of these considerations a few semi-quantitative cyclization (quaternization) experiments were carried out, making use of the area intensities of ^{1}H-NMR signals shown by the mesyl groups in **2** and 2',3'-*O*-isopropylidene-5'-*O*-mesyl-8-bromoadenosine (**2'**) as well as those of the non-covalent mesyl anions after heating these samples (see experimental section). Although Holmes and Robins[9] isolated 2',3'-*O*-isopropylidene-N^3,5'-guanosine cyclonucleoside p-tolylsulfonate (quaternized form) and converted this into 2',3'-*O*-isopropylidene-N^3-5'-cycloguanosine corresponding to **3** in a basic medium, the quaternized form (**iv**, Scheme 2) of **1** or **2** with 8-bromine is not recorded: **2** gave directly **3** in hot water in 5–10 min.[2a] We ourselves did not follow the detailed process of the cyclization of **2** as function of the polarity or basicity of a medium. Therefore, in our case the nature of the mesylate anion from **2** (counterion in **iv** or liberated



Scheme 4.

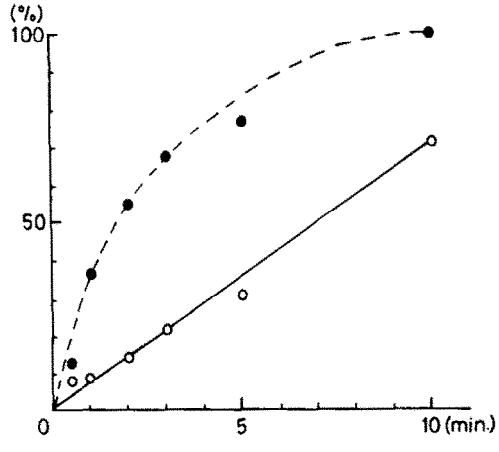


Fig. 1. Intramolecular cyclization of 2',3'-*O*-isopropylidene-5'-*O*-mesyl-8-bromoadenosine (**2'**) (●) and 2',3'-*O*-Isopropylidene-5'-*O*-mesyl-8-bromoguanosine (**2**) (○) in DMSO-d_6 at 90°.

methanesulfonic acid) is not clear. In spite of this mechanistic ambiguity, such a rough cyclization study would be useful for synthetic purpose since no other intramolecular reactions are expected to occur. Figure 1 shows a comparison of the cyclization ratios between the adenosine **2'** and guanosine derivatives **2**. In the neutral medium, cyclization of **2'** is significantly faster than **2**, conforming to the precedent findings.[5-8] Figure 2 indicates the "$_0$ conversion of **2** to **3** *versus* total heating period in different solvents. The calculations are based on the same criteria with the case of Fig 1. It is shown that even in a weakly basic medium (pyridine-d_5/DMSO-d_6, 3:1) the "$_0$ conversion of **2** into **3** was significantly increased as compared with that in DMSO-d_6, substantiating the above-stated mechanistic inspection. The discrepancy observed within 6 min could be attributed to erroneous estimation owing to the weakness of the mesylate anion signal.

Based on these experiments, we decided to use guanosine derivatives protected with methoxymethylene group[10] which might be removed afterwards by

acid hydrolysis. We first attempted to protect **5** with the use of methoxymethylene chloride without success due to the presence of multiple active sites. Accordingly, the amino group was first protected with DMF-acetal to give N^2-dimethylaminomethylidene-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-8-bromoguanine **6**, which with a slight excess methoxymethylene chloride afforded N^2-dimethylaminomethylidene-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-8-bromoguanine **7** in 75% yield. The structures of these compounds were evident on the basis of general spectroscopic data (see Experimental). The location of the methoxymethylene at N^1 in **7** is reflected in the bathochromic shifts of the UV absorptions. **7** was converted to N^2-dimethylaminomethylidene-1- methoxymethylene-9-(2,3-*O*-isopropylidene-5-*O*-mesyl-β-D-ribofuranosyl)-8-bromoguanine **8** in high yield. As expected, compound **8** revealed no indication of intramolecular cyclization after heating in refluxing acetone or in pyridine at 90° for 2 hr. This remarkable reduction in cyclizing tendency seems to suggest that the documented cyclizations[2b] of **1** and **2** are more or less determined by ionization in the base moiety: in these and in our strongly basic conditions direct displacement at C_5· by the ionized base appears to be predominant as shown in Scheme 3, since the N^3,5'-quaternizing cyclizations of guanosine derivatives usually require more forcing conditions.[6,9]

Treatment of **8** with hydrazine (36 fold excess) at room temperature as in the case of **1** and **2** gave 1-methoxymethylene-9-(2,3-*O*-isopropylidene-5-*O*-mesyl-β-D-ribofuranosyl)-8-bromoguanine **9** in practically quantitative yield.[11] It must be noted that the dimethylaminomethylidene group in **8** could not be removed by commonly used ammonolysis.[12] Preliminary trial experiments using **9** or **8** and hydrazine under a variety of conditions confirmed that the 8,5'-*N*-cyclization does occur, but under more forcing conditions than the cyclization of **i**. Thus, in a selected reaction described here, 96 fold excess hydrazine was used under warming to give *ca.* 55% isolated yield of 8,5'-aminimino-1-methoxymethylene-9-(5'-deoxy-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-guanine **10** with minor **4** (8.7%).[13] The structure of **10** was unmistakably confirmed by analysis and spectroscopic data (see Experimental). The *N*-amino group in this compound is sensitive to air-oxidation especially in solution in contrast with the adenosine analog.[14] For the formation of **4**, we assume direct attack by hydrazine at C_6 of **9** and nucleophilic displacement by the resulting 5-amino-imidazole moiety after or before the fragmentatation of the amidine side chain.[13] Acidic deprotection of **10** was examined under several conditions but finally abandoned since the reactions were generally rather complex.[15] Mild oxidation of **10** with sodium metaperiodate smoothly proceeded to give 8,5'-imino-1-methoxymethylene-9-(5'-deoxy-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-guanine (**11a**) in high yield, while the use of iodine pentoxide as in the adenine series[1] led to an uncharacterizable gummy product. Skeletal identity of **11a** with **10** was confirmed by UV spectra. Although in the field of *N*-amino alicyclic amines oxidative deazotation with biradical formation is widely known, we are not aware of such examples of simple and effective deamination probably stemming from the stability of the guanidine type partial structure.[1,16] Reaction of **10** with sodium nitrite yielded first three products different from **10** or **11a**. Two less polar ones were unstable and one of these appeared to be an intermediate for the most polar product, 8,5'-imino-1-methoxymethylene-9-(5'-deoxy-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-xanthine **11b**, which was isolated in a low yield after tedious work-up.[17] On treating **11a** with 90% trifluoroacetic acid, 8,5'-imino-1-methoxymethylene-9-(5'-deoxy-β-D-ribofuranosyl)-guanine **12** formed with a very sparingly soluble product mixture. **12** was hygroscopic but isolable as crystalline hydrochloride. Many other hydrolysis experiments using a variety of mixtures of methanol and concentrated hydrochloric acid resulted in uncharacterizable, sparingly soluble mixtures.

Thus, the major remaining problem in this series of work resides in selection or device of a more suitable protecting group which is base-resistant and removable under milder acidic conditions, and a suitable means of separation.

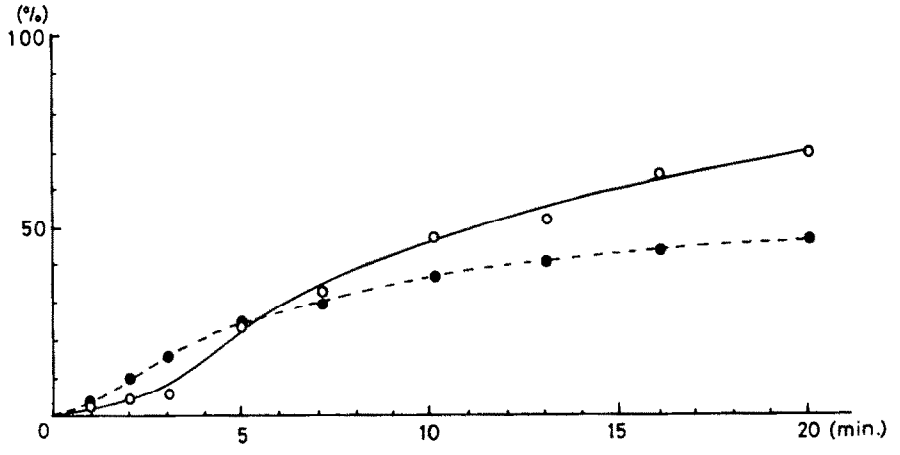


Fig. 2. Intramolecular cyclization of 2',3'-*O*-isopropylidene-5'-*O*-mesyl-8-bromoguanosine (**2**) in DMSO-d_6 (●) and Pyridine-d_5/DMSO-d_6 (3:1) (○) at 90°.

EXPERIMENTAL

The general methods used are similar to those described earlier.[1] Melting points were determined on a Yanagimoto micromelting point apparatus and are not corrected. All evaporations were carried out *in vacuo* at or below 40°.

2′,3′-O-Isopropylidene-5′-O-tosyl-8-bromoguanosine **1**. To a stirred solution of **4** (2.41 g, 5.99 mmol) in pyridine (95 ml) was added at −10 to −5° tosyl chloride (1.485 g, 7.8 mmol). The mixture was then left at 0° for 18 h, treated with water (2 ml) for 20 min at room temp and concentrated to *ca.* 1/5 volume. The solution was poured into ice-water (300 ml) with vigorous stirring and the precipitate collected by filtration. The filtrate was extracted with ethyl acetate (100 ml) and the obtained extract combined with the solid in acetone (30 ml). The acetone solution was again poured into stirred ice-water (300 ml) to give a powdery precipitate, which was collected by suction. The filtrate was extracted with ethyl acetate (2 × 100 ml) and the solution combined with the above solid. After drying over sodium sulfate, the solution was evaporated and the residue digested with a small volume of acetone to give a homogeneous solid. The final crop was obtained by preparative TLC (silica gel, 20% ethanol in benzene). The total solid was dried under high vacuum and recrystallized from acetone at room temp to give 2.7 g (81%) **1** as needles, mp 256–260° (dec.); IR (KBr) 1180 cm^{-1} (covalent sulfonate); UV (MeOH) 225 (ε14000) and 261 nm (ε17900). (Found: C, 43.33; H, 4.15; N, 12.29. Calc. for $C_{20}H_{22}N_5O_7SBr$: C, 43.18; H, 3.99; N, 12.59%).

Reaction of **1** *with hydrazine*. (A). A mixture of **1** (1.11 g, 2 mmol) and 100% hydrazine monohydrate (2 ml, 40 mmol) in methanol (15 ml) was stirred under ice-cooling for 1 h and then at room temp.

After 3.5 h from the beginning of the reaction, crystalline solid began to deposit. After totally 5.5 h (TLC at this stage using silica gel and 20% ethanol in benzene showed the presence of a main polar product with two minor faster-moving substances, more polar one of which was the starting material), the mixture was cooled with ice, the solid filtered and washed with a small volume of methanol to give TLC-pure **3** (326 mg). The filtrate was evaporated and the residue repeatedly co-evaporated with ethanol. The obtained brown paste was then thoroughly evaporated under high vacuum to remove the residual hydrazine. Trituration of the residue with a small volume of cold methanol gave another crop of **3** (107 mg), which was collected by suction. The filtrate was again evaporated and the residue partitioned between chloroform (40 ml) and water (7 ml) to give an additional crop (105 mg) of **3** as insoluble precipitate (total 543 mg, 67.5%, TLC-pure). For analysis a part was recrystallized from a mixture of water and methanol to give colorless crystals **3**, which gradually decomposed above 250° but did not melt below 300°. UV $\lambda_{max}^{0.01N\ HCl\ MeOH}$ 252 nm (ε14500); λ_{max}^{MeOH} 221 (ε26300) and 266 nm (ε13500); $\lambda_{max}^{0.01N\ NaOH\ MeOH}$ 221 (ε23100) and 265 nm (ε13000) [lit.[2a]: λ_{max} (pH 1) 256 nm (ε16700); λ_{max} (H_2O) 220 (ε26500) and 270 nm (ε14500); λ_{max} (pH 13) 251 (sh) and 270 nm (ε10100)]; ^{1}H-NMR (DMSO-d_6) δ 1.29, 1.49 (each 3H, isopropylidene), 3.88 (1H, dd, $J_{5'a,b}$ = 14 Hz, $J_{5'a,4'}$ = 2 Hz, $H_{5'a}$), 4.63 (1H, dd, $J_{5'b,a}$ = 14 Hz, $J_{5'b,4'}$ = 3 Hz, $H_{5'b}$), 4.69 (1H, d, $H_{2'}$ or $H_{3'}$, $J_{2',3'}$ = 6 Hz), 4.86 (1H, m, $H_{4'}$), 5.01 (1H, d, $H_{3'}$ or $H_{2'}$, $J_{3',2'}$ = 6 Hz), 6.10 (1H, s, $H_{1'}$) and 7.18 (2H, br s, D_2O-exchangeable, NH_2). (Found: C, 38.60; H, 4.08; N, 17.33. Calc. for $C_{13}H_{14}N_5O_4Br{\cdot}H_2O$: C, 38.82; H, 4.01; N, 17.41%).

This compound was shown to be identical with an authentic sample[2a] by IR spectra.

(B): A mixture of **2** (797 mg, 1.77 mmol) and 100% hydrazine monohydrate (1.77 ml, 35.5 mmol) in methanol (15 ml) was stirred at room temperature for 67 h, during which time TLC analysis was repeated using solvent mixtures, 20% ethanol in benzene and 20% methanol in chloroform. Precipitation of **3** was observed at the early stage of the reaction as in procedure A, but the mixture became again clear during that time. TLC at this stage showed at least five spots, of which one main spot corresponded to the least polar (faster-running than the starting material) and another main one to the most polar product (more polar than **3**). **3** was also detected in minor quantity. The mixture was thoroughly evaporated in the same way with procedure (A), and the residue partitioned between chloroform (30 ml) and water (7 ml). The separated chloroform solution was filtered to remove a small amount of insolubles, dried and evaporated to give a gum containing the least polar main product. On attempted TLC-purification, about half of this product changed to a more faster-moving substance. Hence, the recovered two-component mixture as a gum was left at room temp for 2 days to give crystals, which were collected and recrystallized from methanol at room temp to afford 30 mg (4.52%) of **4**, mp 223–226°; UV (MeOH) 277 nm (ε8800); ^{1}H-NMR ($CDCl_3$) δ 1.35, 1.55 (each 3H, s, isopropylidene), 3.33 (2H, m, 5′-methylene), 4.63 (2H, m, $H_{4'}$ and $H_{2'}$ or $H_{3'}$, $J_{2',3'}$ = 6 Hz), 4.92 (1H, d, $H_{3'}$ or $H_{2'}$, $J_{2',3'}$ = 6 Hz), 5.96 (1H, s, $H_{1'}$) and 6.36 (1H, d, NH-bridge, D_2O-exchangeable). Signals for the carbohydrazido group did not appear clearly. (Found: C, 38.34; H, 4.36; N, 18.45. Calc. for $C_{12}H_{16}N_5O_4Br$: C, 38.52; H, 4.31; N, 18.72%).

The filtrate of **4** was evaporated and the residue heated in acetone (10 ml) to reflux for 1 h. After evaporation, the main product was isolated by preparative TLC ($CHCl_3$:MeOH, 9:1) and recrystallized from acetone to give 120 mg (0.29 mmol, 16.4%) of **4′** as crystals of mp 215–217°; UV (MeOH) 224 (ε17200) and 287 nm (ε22800); ^{1}H-NMR ($CDCl_3$) δ 1.35, 1.56 (each 3H, s, isopropylidene), 1.98, 2.12 (each 3H, methyls of acetone hydrazone), 3.36 (2H, m, 5′-methylene), 4.64 (2H, m, $H_{4'}$ and $H_{2'}$ or $H_{3'}$, $J_{2',3'}$ = 6 Hz), 4.94 (1H, d, $H_{3'}$ or $H_{2'}$, $J_{3',2'}$ = 6 Hz), 5.98 (1H, s, $H_{1'}$), 6.50 (1H, br s, imino-bridge, D_2O-exchangeable) and 9.21 (1H, br s, amido-NH, D_2O-exchangeable). (Found: C, 43.43; H, 4.87; N, 16.97. Calc. for $C_{15}H_{20}N_5O_4Br$: C, 43.49; H, 4.87; N, 16.91%).

Cyclization experiments with 2′,3′-O-Isopropylidene-5′-O-mesyl-8-bromoadenosine **2′** *and 2′,3′-O-Isopropylidene-5′-O-mesyl-8-bromoguanosine* **2**. *(1) Comparison of the Cyclizing Propensity of* **2′** *and* **2** *(Fig 1)*. A solution of **2** (25 mg) in DMSO-d_6 (0.33 ml) in a sealed NMR tube was submitted to ^{1}H-NMR measurement at room temperature. The tube was then heated at 90° for 30 sec and immediately frozen at −20°. before the second ^{1}H-NMR measurement; measurements with the same sample were conducted after similar treatments adopting several heating periods such as 1, 2, 3 min, etc. The sample was usually kept in frozen state at −20° except when the heating and ^{1}H-NMR recording were carried out. In the case of **2′** a solution of this compound (50 mg) in DMSO-d_6 (0.3 ml) was submitted to similar treatments and PMR measurements. As to **2**, the evaluation of % conversion after each total heating time was based on the area integrals for the resonance of mesyl anion at 2.41 ppm (Peak 2) and that of the mesyl group of the starting material **2** at 3.08 ppm (Peak 1). Thus, the values obtained from equation: $\frac{(\text{Peak 2})}{(\text{Peak 1}) + (\text{Peak 2})} \times 100$ (%) were plotted against the total heating time. For the adenosine derivative **2′** the corresponding resonance at 2.30 ppm (Peak 2) and 3.08 ppm (Peak 1) were used. It is to be noted that only one kind of mesyl anion peak was detected through this experiment.

(2) *Time-dependent Cyclization Ratios of* **2** in DMSO-d_6 and Pyridine-d_5:DMSO-d_6 *(3:1) at 90° (Fig 2)*. The evaluation of % conversion after each total heating period was done in the same way as the above procedure 1, using Peak 1 at 3.08 ppm and Peak 2 at 2.39 ppm in DMSO-d_6 (concentration: 42 mg/ml) and the corresponding peaks at 3.31 and 2.95 ppm in the mixed solvent (concentration: 42.5 mg/ml). The reason for the use of the mixed solvent was the rather limited solubility of **2** in pyridine-d_5.

N^2-Dimethylaminomethylidene-9-(2,3-O-isopropylidene-β-D-ribofuranosyl)-8-bromoguanine **6**. To a solution of **5** (9 g,

22.4 mmol) in DMF (110 ml) was added *N,N*-dimethylformamide dimethylacetal (DMF-acetal) (11.2 ml, 112 mmol) and the mixture left at room temp for 20 h. After evaporation, the residue was thoroughly digested with ice-water (100 ml) and the solid collected by suction. Recrystallization from a 9:1 mixture of acetone and water gave 8.92 g (90.7%) of **6** as colorless needles, mp 234–235.5°; UV (MeOH) 235 (ε14600) and 305 nm (ε24100); ^{1}H-NMR (DMSO-d_6) δ 1.32, 1.52 (each 3H, s, isopropylidene), 3.02, 3.15 (each 3H, s, methyl of the dimethylaminomethylidene), 3.50 (2H, dd-like t, 5-CH_2, $J_{5,OH}$ = 6 Hz, changed to d with J = 6 Hz on D_2O addition), 4.10 (1H, m, H_4), 4.95 (2H, m, reduced to one proton dd on D_2O addition, $J_{2,3}$ = 6 Hz, $J_{2,1}$ = 3 Hz, $J_{5,OH}$ = 6 Hz, H_2 and 5-OH), 5.56 (1H, dd, $J_{3,2}$ = 6 Hz, $J_{3,4}$ = 2.5 Hz, H_3), 5.90 (1H, d, $J_{1,2}$ = 3 Hz, H_1), 8.52 (1H, s, >N-CH=) and 11.42 (1H, s, lactam NH, D_2O-exchangeable). (Found: C, 42.14; H, 4.65; N, 18.40. Calc. for $C_{16}H_{21}N_6O_5Br$: C, 42.02; H, 4.63; N, 18.38%). 0.35 g (3.9%) of the starting material was recovered.

N^2-*Dimethylaminomethylidene*-1-*methoxymethylene*-9-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)-8-*bromoguanine* **7**. Compound **6** (9.45 g, 20.66 mmol) was dissolved in DMF (75 ml) by slight warming. After cooling the mixture up to room temp, 50% oil-immersed sodium hydride (992 mg, 20.67 mmol) was added in several portions under stirring. After stirring at room temp for 30 min, methoxymethyl chloride (1.57 ml, 20.66 mmol) was added and the mixture stirred for additional 1 h. Further methoxymethyl chloride (0.2 ml, total 1.13 fold excess) was added and the mixture stirred for additional 8 h, at the end of which time the mixture indicated a pH of around 6. After evaporation, the mixture was partitioned between chloroform (100 ml) and water (100 ml). The aqueous phase was again extracted with chloroform (2 × 70 ml). The combined chloroform solution was dried over sodium sulfate, concentrated to *ca.* 30 ml and applied on a silica gel column (4 × 40 cm). Elution with $CHCl_3$/MeOH (9:1) gave the first main fraction as a gum, which crystallized on rubbing in a small volume of acetone. Recrystallization from acetone gave 7.21 g (75.2%) of **7** as colorless needles, mp 224–226°; UV (MeOH) 238 (ε12800), 280 (ε13000, sh) and 313 nm (ε22500); ^{1}H-NMR ($CDCl_3$) δ 1.38, 1.64 (each 3H, s, isopropylidene), 3.13, 3.21 (each 3H, s, methyl of the dimethylaminomethylidene), 3.42 (3H, s, methyl of the methoxymethylene), 3.83 (2H, m, 5-CH_2), 4.35 (1H, m, 5-OH, D_2O-exchangeable), 4.37 (1H, m, $J_{3,4}$ = 2 Hz, H_4), 4.98 (1H, dd, $J_{3,2}$ = 6 Hz, $J_{3,4}$ = 2 Hz, H_3), 5.30 (1H, dd-like t, $J_{2,3}$ = 6 Hz, $J_{1,2}$ = 5 Hz, H_2), 5.68 (2H, s, methylene of the methoxymethylene), 5.98 (1H, d, $J_{1,2}$ = 5 Hz, H_1) and 8.37 (1H, s, methine of the dimethylaminomethylidene). (Found: C, 42.86; H, 4.97; N, 17.08. Calc. for $C_{18}H_{25}N_6O_6Br$: C, 43.12; H, 5.03; N, 16.76%). 0.7 g (7.4%) of the starting material was recovered from the second fraction.

N^2-*Dimethylaminomethylidene*-1-*methoxymethylene*-9-(2,3-O-*isopropylidene*-5-O-*mesyl*-β-D-*ribofuranosyl*)-8-*bromoguanine* **8**. To a stirred ice-cold solution of **7** (5.75 g, 11.47 mmol) in pyridine (170 ml) was added methanesulfonyl chloride (1.07 ml, 13.82 mmol) in small portions.

After stirring at room temperature for 1 h, the mixture was left at 0° for 15 h and then treated with methanol (10 ml) for 30 min at room temperature. After evaporating the solvent, water (100 ml) was added to the residue and the mixture extracted with ethyl acetate (2 × 75 ml). The combined ethyl acetate solution was dried, evaporated and the residue brought to crystallization with a small amount of methanol to give 5.84 g (88%) of **8** as homogeneous crystals, mp 165–168°: IR (KBr) 1180 cm^{-1} (covalent sulfonate); ^{1}H-NMR ($CDCl_3$) δ 1.40, 1.62 (each 3H, s, isopropylidene), 2.89 (3H, s, mesyl), 3.17, 3.22 (each 3H, s, methyl of the dimethylaminomethylidene), 3.44 (3H, s, methyl of the methoxymethylene), 4.38 (3H, br s, H_4 and 5-CH_2), 5.08 (1H, m, $J_{2,3}$ = 6 Hz, H_3), 5.60 (1H, dd, $J_{2,3}$ = 6 Hz, $J_{2,1}$ = 2 Hz, H_2), 5.73 (2H, s, methylene of the methoxymethylene), 6.10 (1H, d, $J_{1,2}$ = 2 Hz, H_1) and 8.47 (1H, s, methine of the dimethylaminomethylidene). (Found: C, 39.39; H, 4.78; N, 14.42. Calc. for $C_{19}H_{27}N_6O_8SBr$: C, 39.39; H, 4.70; N, 14.51%).

1-*Methoxymethylene*-9-(2,3-O-*isopropylidene*-5-O-*mesyl*-β-D-*ribofuranosyl*)-8-*bromoguanine* **9**. Compound **8** (50 mg, 0.086 mmol) was dissolved in methanol (1 ml) by slight warming. After cooling to room temp, 100% hydrazine monohydrate (0.15 ml, 3.1 mmol) was added and the mixture left at room temp. After 6 h, the precipitation of a voluminous solid was noted. After standing at room temperature overnight, 41 mg (90.6%) of TLC-pure crystals **9** were collected and recrystallized from acetone for analysis, mp 185–187°; UV (MeOH) 261 (ε12900) and 280 nm (ε9100, sh); IR (KBr) 1180 cm^{-1} (sulfonate ester); ^{1}H-NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 1.40, 1.58 (each 3H, s, isopropylidene), 3.03 (3H, s, mesyl), 3.36 (3H, s, methyl of the methoxymethylene), 4.41 (3H, br s, H_4 and 5-CH_2), 5.43 (4H, br s, H_2, H_3 and methylene of the methoxymethylene), 6.02 (1H, s, H_1) and 7.11 (2H, br s, NH_2, D_2O-exchangeable). (Found: C, 36.68; H, 4.22; N, 13.34. Calc. for $C_{16}H_{22}N_5O_8SBr$: C, 36.65; H, 4.23; N, 13.36%).

8,5′-*Aminimino*-1-*methoxymethylene*-9-(5′-*deoxy*-2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)-*guanine* **10**. Compound **8** (5.60 g, 9.66 mmol) was dissolved in methanol (230 ml) by slight warming and the solution cooled up to room temp. 100% hydrazine monohydrate (45 ml, 0.926 mmol, 96 fold) was added and the mixture left at room temp for 20 h. TLC at this stage showed the presence of **9** as major product and no starting material. The mixture was then heated at 60–65° for 15 h, during which time almost all of **9** disappeared as judged by TLC. The mixture was cooled up to room temp, evaporated and the residue repeatedly co-evaporated with ethanol to give a brown paste, which was partitioned between chloroform (150 ml) and water (100 ml). The separated aqueous layer was again extracted with chloroform (150 ml + 3 × 100 ml). The combined chloroform solution was dried, evaporated and the residue dissolved in a minimum amount of a mixture of chloroform and methanol by slight warming. On seeding or agitation by a spatula, the cooled solution gave crystals **10** which were collected. The filtrate was evaporated and the residue applied on a silica gel column (2.8 × 20 cm). Elution with $CHCl_3$/MeOH (9:1) gave from the first minor fraction 315 mg (8.7%) of **4**. The second main fraction gave another crop of **10**. Recrystallization of the main product from MeOH/$CHCl_3$ (9:1) gave 2.0 g (54.5%) of colorless needles **10**, mp 272–275°: UV (MeOH) 259.5 (ε17100) and 287 nm (ε8500); ^{1}H-NMR (DMSO-d_6) δ 1.27, 1.43 (each 3H, s, isopropylidene), 3.25 (4H, m, overlapped resonances of $H_{5'a}$ and the methyl of the methoxymethylene), 3.50 (1H, dd, $J_{5'a,b}$ = 13 Hz, $J_{5'b,4'}$ = 3 Hz, $H_{5'b}$), 4.54 (1H, d, $J_{4',5'b}$ = 3 Hz, $H_{4'}$), 4.57 (1H, d, $J_{2',3'}$ = 6 Hz, $H_{2'}$ or $H_{3'}$), 4.93 (1H, d, $J_{3',2'}$ = 6 Hz, $H_{3'}$ or $H_{2'}$), 4.34–5.17 (2H, very shallow signal, >N-NH_2, D_2O-exchangeable), 5.35 (2H, s, methylene of the methoxymethylene), 5.88 (1H, s, $H_{1'}$) and 6.85 (2H, br s, 2-NH_2, D_2O-exchangeable). (Found: C, 47.53; H, 5.60; N, 25.54. Calc. for $C_{15}H_{21}N_7O_5$: C, 47.49; H, 5.58; N, 25.84%).

8,5′-*Imino*-1-*methoxymethylene*-9-(5′-*deoxy*-2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)-*guanine* **11a**. To a stirred solution of **10** (1 g, 2.64 mmol) in a 1:1 mixture (160 ml) of methanol and DMF was added sodium metaperiodate (677 mg, 3.17 mmol, 1.2 fold). After 1 h, the mixture was evaporated and the residue extracted with chloroform (5 × 100 ml) in the presence of water (100 ml). The combined chloroform solution was dried, evaporated and the residue digested with methanol (25 ml) to give crystals **11a**, which were collected. The filtrate was evaporated and the residue purified by column chromatography [silica gel, 2 × 17 cm, $CHCl_3$/MeOH (9:1)] to give another crop. Recrystallization of the total product from MeOH/$CHCl_3$ (2:1) afforded 790 mg (82.3%) of **11a** as colorless plates, mp above 300°: UV (MeOH) 260 (ε18600) and 292.5 nm (ε9000); ^{1}H-NMR (DMSO-d_6) δ 1.26, 1.42 (each 3H, s, isopropylidene), 2.95–3.34 (2H, m, 5′-CH_2), 3.25

(3H, s, methoxyl), 4.57 (1H, d, $J_{2',3'} = 6$ Hz, $H_{2'}$ or $H_{3'}$), 4.58 (1H, d, $J_{4',5'} = 3$ Hz, H_4), 4.83 (1H, d, $J_{3',2'} = 6$ Hz, $H_{3'}$ or $H_{2'}$), 5.33 (2H, s, methylene of the methoxymethylene), 5.84 (1H, s, H_1), 6.68 (1H, d, $J = 4$ Hz, imino-bridge, D_2O-exchangeable) and 6.82 (2H, br s, 2-NH_2, D_2O-exchangeable). (Found: C, 49.33; H, 5.53; N, 23.18. Calc. for $C_{15}H_{20}N_6O_5$: C, 49.44; H, 5.53; N, 23.07%).

8,5'-*Imino*-1-*methoxymethylene*-9-(5'-*deoxy*-2',3'-*O*-*isopropylidene*-β-D-*ribofuranosyl*)-*xanthine* **11b**. To a ice-cooled stirred solution of **10** (200 mg, 0.527 mmol) in 80% acetic acid (10 ml) was added sodium nitrite (261 mg, 3.69 mmol). After the solid salt dissolved, the mixture was left at 0° for 16.5 h and then at room temperature for 9.5 h. TLC at this stage indicated three faster-moving products (A, B and C in the order of polarity decrease: B was the main product). The mixture was then evaporated, co-evaporated with ethanol and the residue digested with a small volume of ice-water. The sparingly soluble solid was filtered and the aqueous filtrate extracted with ehyl acetate. The ethyl acetate extract was combined with the above solid (TLC-pattern was unchanged at this stage), applied on a silica gel column (10 × 20 cm) and eluted with $CHCl_3$/MeOH (9:1). Comparative TLC-analysis with all the eluants indicated that the major part of B changed to the most polar A, rendering the complete isolation of A and B difficult. However, the mixture of A and B gave crystals (A) on standing as a saturated ethanolic solution. These were collected and recrystallized from ethanol to afford 25 mg (12.6%) of **11b**, mp above 300°: UV (MeOH) 209 (ε24300), 253.5 (ε 16500) and 303.5 (ε8400); ^{1}H-NMR ($CDCl_3$/DMSO-d_6, 4:1) δ 1.32, 1.51 (each 3H, s, isopropylidene), 3.26 (3H, s, methoxyl), 3.1-3.4 (2H, m, 5'-CH_2), 4.72 (4H, m, methylene of the methoxymethylene, H_4 and H_2 or $H_{3'}$), 4.91 (1H, d, $J_{3',2'} = 6$ Hz, H_3 or H_2), 5.99 (1H, s, H_1), 6.73 (1 H, br s, imino-bridge, D_2O-exchangeable) and 8.78 (1 H, br s, N^3H, D_2O-exchangeable). (Found: C, 49.31; H, 5.50; N, 18.41. Calc. for $C_{15}H_{19}N_5O_6 \cdot 1/4\,C_2H_5OH$: C, 49.40; H, 5.48; N, 18.58%). Collection of subsequent crops was abandoned mainly due to the limited solubility and high polarity of the mixture, which made application to chromatography difficult. On the other hand, the separated impure C decomposed into a complex mixture on attempted purification by preparative TLC.

Hydrochloride of 8,5'-*Imino*-1-*methoxymethylene*-9-(5'-*deoxy*-β-D-*ribofuranosyl*)*guanine* **12**. A solution of **11a** (300 mg, 0.823 mmol) in 90% CF_3CO_2H (18 ml) was left at room temperature for 21.5 hr and then evaporated. The residue was repeatedly co-evaporated with ethanol, dissolved in methanol (60 ml) and neutralized with anion exchange resin, IRA-93 (OH-form). The resin was filtered, washed with methanol (400 ml) and the combined methanolic solution evaporated to give a solid residue, which was taken into a mixture of chloroform and methanol and the insoluble material filtered off. The soluble part was submitted to preparative TLC [20 × 20 cm, silica gel, $CHCl_3$/MeOH (8:2] and the main fraction eluted with $CHCl_3$/MeOH (9:1) to give a very hygroscopic powder. This was dissolved in methanol (50 ml), acidified with a few drops of saturated solution of hydrogen chloride in dioxane and the mixture immediately evaporated to give a powder. After repeated co-evaporation with ethanol, the solid was dissolved in ethanol by slight warming and filtered with Norite. The solution was concentrated up to saturation and left at room temperature to give very gradually powder-like crystals (32 mg, 11.3%), mp above 300°: UV (MeOH) 259.5 (ε 17300) and 292 nm (ε 7800); ^{1}H-NMR (DMSO-d_6) δ 3.03-3.55 (2H, m, 5'-CH_2), 3.27 (3H, s, methoxyl), 4.07 (1H, d after D_2O-addition, $J_{2',3'} = 6$ Hz, $H_{2'}$ or $H_{3'}$), 4.24 (1H, d after D_2O-addition, $J_{3',2'} = 6$ Hz, H_3 or $H_{2'}$), 4.47 (1H, m, $H_{4'}$), 5.0 5.5 (2H, m, 2'- and 3'-OH, D_2O-exchangeable), 5.35 (2H, br s, methylene of the methoxymethylene), 5.89 (1H, s, H_1), 7.22 (2H, br s, 2-NH_2, D_2O-exchangeable) and 7.72 (1H, br s, imino-bridge, D_2O-exchangeable). (Found: C, 41.97; H, 4.97; N, 24.21. Calc. for $C_{12}H_{16}N_6O_4 \cdot HCl$: C, 41.81; H, 4.97; N, 24.38%).

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