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Methylation of desmethyl analogue of Y nucleosides. Wyosine from guanosine[1]

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ABSTRACT

Wyosine 1a, one of the fluorescent hypermodified Y nucleosides found in tRNAs^{Phe}, was synthesized chemically from its biogenetic precursor guanosine 2. The route involved transformation of 2 into the tricyclic structure 3a and subsequent methylation at N-4. The major products of various methylation procedures were isomers of wyosine, methylated at N-5 (3b) or at N-1 (4). Mesoionic compound 4 is a new analogue of 7-methylguanosine 5, modified nucleoside occuring in the unique positions in transfer, messenger and ribosomal RNAs. The chromatographic and spectral characteristics of wyosine and its isomers is given.

INTRODUCTION

Hypermodified, fluorescent Y bases and respective nucleosides (1a-d), occuring in tRNAs specific for phenylalanine have been the subject of many studies /2-5/ due to their distinctive physical and chemical properties as well as biological interest.

A multistep synthesis of wyosine (nucleoside Y_t, 1a) starting from 5-(methylamino) -1-β-D-ribofuranosylimidazole-4-carboxamide has been reported /6/ and recently improved /7/. Since it has been shown that biosynthetically, Y nucleosides are derived from guanosine /8,9/, the possibility of an analogous chemical transformation was of interest.

Alkylation at the N-3 position has not been noted, even in traces, in any of the numerous papers /10-15/ on alkylation of guanosine and deoxyguanosine. Therefore we approached the guanosine to wyosine conversion via addition of the third ring to form 4-desmethylwyosine /16/ - 5,9-dihydro-6-methyl-9-oxo-3-(β-D-ribofuranosyl)5H-imidazo [1,2-a] purine, 3a, and subsequent methylation. In addition to wyosine, its isomer, methylated at the N-1 position (4), also deserved attention as an analogue of 7-methylguanosine 5, a unique component of transfer /17,18/, messenger /19/ and ribosomal /20/ RNAs, which is known to exist as a zwitterion at physiological pH.

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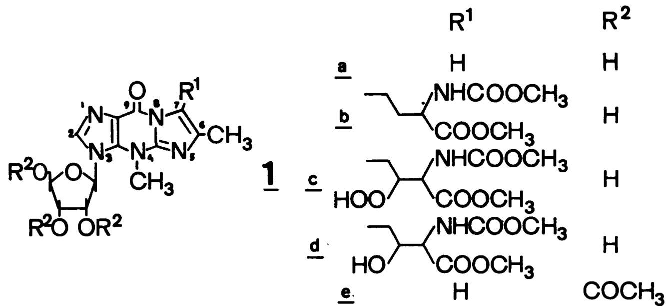

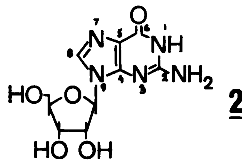

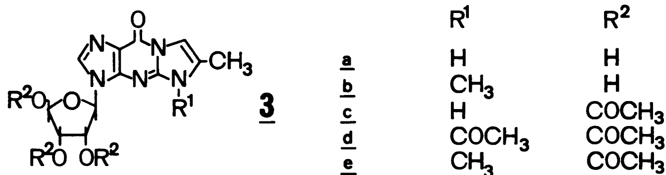

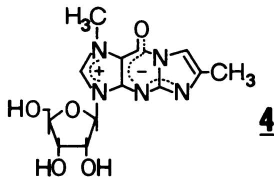

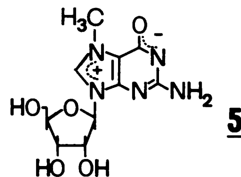

The literature data on methylation of 3a have been limited to the report of Kasai et al. /2/, who after reacting dimethylsulfoxide-methanolic solution of 3a with ethereal diazomethane, isolated exclusively 5-methyl derivative 3b.

This paper describes our methylation studies on 4-desmethylwyosine, aimed especially towards N-4 and N-1 derivatives.

RESULTS AND DISCUSSION

On reinvestigation of literature methylation experiment /2/ on 4-desmethylwyosine 3a we found a trace of flourescent material, which partly decomposed to wye base on attempted isolation. To increase the stability of the glycisidic bond, 3a was transformed into 2', 3', 5'- tri-O-acetyl deriv-

ative 3c. Of additional advantage was decreased polarity of 3c in comparison with that of parent nucleoside 3a, which gave new possibilities for the methylation medium and chromatographic separations.

The behaviour of 4-desmethylwyosine in acetylation and deacetylation reactions was distinctly different from that of guanosine. The conditions required for a complete acetylation of 3a were very mild. Acetic anhydride in pyridine at room temperature transformed it smoothly and almost quantitatively into 5-N,2'-O,3'-O, 5'-O-tetra-acetyl compound 3d, whereas tetraacetylation of guanosine could have been accomplished only at the reflux temperature /21/. N-acetyl linkage of 3d was more labile than O-acetyl ones and was easily and selectively split with pyridine-methanol-water to give 3c.

Methylation of triacetate 3c with diazomethane in dichloromethane solution did not result in essentially changed methylation pattern in comparison with that of unblocked nucleoside in dimethyl sulfoxide-methanol-ether. The major product as being N-5 methylated 3e was identified on the basis of the UV and ^{1}H NMR spectra and by the deacetylation reaction which gave 3b. In the present experiment however, two fluorescent minor methylation products were found. They were successfully separated from each other and from preponderant N-5 methyl derivative by short column chromatography on silica gel. One of them, isolated in 3% yield was identical with wyosine triacetate 1e, described by Goto et al. /6/, with respect to the ^{1}H NMR spectrum. Its UV spectrum showed two maxima λ_{max}^{MeOH} 236 (ε34,300) and 292 nm (ε 8,000), which differed from Goto's results - λ_{max}^{MeOH} 234 (ε17,300) and 288 nm (ε 5,300) but were very close to that given recently /7/ for pure, crystalline wyosine. The structure of the other fluorescent compound, which we call tentatively "pseudowyosine" has not been yet completely identified. Treatment of triacetate 1e with 14N methanolic ammonia at room temperature for various RNAs.

In the above search for the methylation procedures, most favourable for the formation of N-4 and N-1 methylated tricyclic nucleosides, chromatographic, UV and ^{1}H NMR data shown in TABLES 2-4 were of important assistance.

It is worthy to note that although the R_F values on TLC of wyosine triacetate and its congeners are very close, it was possible to separate these compounds using short column technique. Although the best separation on TLC was achieved in the solvent system B , chloroform-methanol (9:1), for short 2.5 hours resulted in wyosine 1a, which crystallized out from the deblocking medium. The properties of wyosine thus obtained are listed in TABLE 1 together with respective data reported by Itaya et al. /7/. The comparison showed

TABLE 1. Properties of synthetic wyosine.

	Wyosine from guanosine	Wyosine according to /7/
Melting point, °C	231 decomp.	235 decomp.
Half time of hydrolysis of the glycosidic bond at pH 2.9, 37°C		
$t_{1/2}$, min	55	41
UV (H_2O)	pH 6.8	pH 7
λ_{max}, nm	236 (ε 34,500) 296 (ε 8,100)	236 (ε 34,700) 296 (ε 8,000)
Fluorescence (H_2O)		not reported
Emission λ_{max}, nm	431 (excitation at 305 nm)	
Excitation λ_{max}, nm	305 (emission at 431 nm)	
^1H NMR $[(CD_3)_2SO]$		
ppm, δ		
2-H	8.25	8.25
7-H	7.39	7.37
N-4CH_3	4.09	4.09
6-CH_3	2.23	2.23
^{13}C NMR $[(CD_3)_2SO]$ /22/		not reported
ppm from TMS		
6-C	137.21	
7-C	105.62	
N-4 CH_3	33.81	
6-CH_3	14.03	

practical identity of the characteristic features of the nucleoside obtained by two entirely different routes. Our results also confirmed the stability of wyosine under neutral conditions.

Neither of diazomethane methylation reactions of 4-desmethylwyosine 3a discussed above resulted in significant substitution at the N-1 position. That is in distinct contrast to guanosine which reacts with diazomethane at N-7 /10,15/, the position corresponding to N-1 of 3a. Methylation agents of the type MeX, however, similarly to guanosine, reacted with 3a at N-1 to give

TABLE 2. Thin Layer Chromatography

Compound	R_F values x 100 in systems [a]					
	A	B	C	D	E	F
3c	77	51	68	84	-	-
3d	89	74	83	88	-	-
3e	83	63	73	81	-	-
1e	85	67	79	83	-	-
ie "pseudo"	87	74	83	86	-	-
3a	26	-	-	84	58	66
3b	29	-	-	76	51	61
1a	25	-	-	82	58	59
4	02	-	-	33	40	-
2	02	-	-	78	51	50
5	01	-	-	15	38	-

[a] see EXPERIMENTAL

4, an analogue of 7-methylguanosine 5. Dimethyl sulfate was superior to methyl iodide in this respect because of the higher reaction rate and easier transformation of the salt, formed originally in the reaction, into the zwitterionic nucleoside. Verification of the site of methylation as being N-1 resulted from the independent synthesis of 4 by formation of the addit-

TABLE 3. Ultraviolet Spectra

Compound	λ_{max} nm ($\varepsilon \times 10^{-3}$)						Solvent
3c	229	(33.7)	285	(14.5)			a
3d	223	(40.0)	275	(11.2)	303	(12.7)	a
3e	230	(34.6)	286	(16.4)			a
1e	236	(34.3)	292	(8.0)			a
1e "pseudo"	230		287				a
3a	231	(35.8)	295	(13.9)			b
3b	233	(34.7)	289	(13.1)			b
1a	236	(34.5)	296	(8.1)			b
4	229	(23.0)	279	(7.6)	303	(6.6)	b

[a] 95% EtOH; [b] H_2O, pH 6.8

TABLE 4. 90 MHz ^{1}H NMR spectra

Cpd	Chemical shifts of the protons in ppm (δ) from TMS [a]													
	2	7	N-R	$6-CH_3$	1'	2'	3'	4'	5'	2'-OH[b]	3'-OH[b]	5'-OH[b]	O-acetyl groups		
3c	7.78 s,1[c]	7.35 d,1	10.11[b] br,1	2.37 d,3	6.15—6.00 m,2		5.78 m,1	4.58—4.40 m,3					2.14 s,3	2.09 s,3	2.05 s,3
3d	7.96 s,1	7.45 d,1	2.98 s,3	2.55 d,3	6.13—5.93 m,2		5.42 t,1	4.51—4.30 m,3						2.13 s,9	
3e	7.70 s,1	7.43 d,1	3.68 s,3	2.34 d,3	6.20——5.91 m,3			4.55—4.33 m,3					2.14 s,3	2.12 s,3	2.06 s,3
1e	7.74 s,1	7.38 d,1	4.19 s,3	2.32 d,3	6.27 d,1	5.87 t,1	5.50 t,1	4.51 q,1	4.31 d,2				2.18 s,3	2.16 s,3	2.10 s,3
3a	8.13 s,1	7.36 d,1	12.47[b] br,1	2.27 d,3	5.82 d,1	4.49 q,1	4.13 q,1	3.92 q,1	3.64 br,2	5.42 d,1	5.23—5.00 m,2				
3b	8.13 s,1	7.46 s,1	3.58 s,3	2.32 d,3	5.87 d,1	4.60 q,1	4.17 q,1	3.94 q,1	3.60 br,2	5.43 d,1	5.20 d,1	5.03 t,1			
1a	8.25 s,1	7.39 d,1	4.09 s,3	2.23 d,3	6.14 d,1	4.46 q,1	4.20—3.90 m,2		3.63 br,2	5.71 d,1	5.32 d,1	5.13 t,1			
4	9.24[b] s,1	7.21 d,1	4.06 s,3	2.17 d,3	5.85 d,1	firm assignment could not be made									

[a] In $CDCl_3$ for cpds 3c, 3d, 3e, 1e; in $(CD_3)_2SO$ for cpds 3a, 3b, 1a, 4.

[b] Removed by addition of D_2O.

[c] Figures following the observed multiplicity of signals represent number of protons, as estimated by integration.

ional ring onto 7-methylguanosine 5. Treatment of 5 in anhydrous dimethylformamide solution with potassium carbonate, followed by bromoacetone, at room temperature provided the compound in all respects identical with the methylation product of 3a with dimethyl sulfate.

1-Methyl-4-desmethylwyosine 4 underwent easily base-included hydrolytic opening of the imidazolium ring, characteristic for 7-methylguanosine, and other 7-substituted purine nucleosides /23/. The ring opening of 4 was monitored by the UV spectra in which the increase in absorbance at 234 nm corresponded to the formation of the ring-opened product. It has been postulated /24/ that the rate of ring opening reflects the extent of zwitterionic character i.e. the presence of the negative charge in the pyrimidine moiety. At pH 10 compound 4 was hydrolyzed in the imidazolium ring with $t_{1/2}$ of 13 min, a value in between of these reported /24/ for 7-methlguanosine and 7-methylinosine (25 and 5 min respectively). The similarity of chemical properties of tricyclic 4 and those of 7-methylguanosine was also demonstrated at ^{1}H NMR spectrum. The C-2 proton in 4, analogously to the C-8 proton in 7-methylguanosine, resonated at low field, at 9.24 δ and exchanged readily with D_2O at room temperature.

The mesoionic character of 4, close to that of 7-methylguanosine, together with an extra ring binding two nitrogen centers and changing the dimensions of the molecule suggest this analogue as an interesting candidate for investigation of the essence of the function of 7-methylguanosine in column system dichloromethane-ethanol gave the optimal results. The differences in the UV and NMR spectra provided sensitive test for the purity of the isolated compounds.

The experiments on changing the methylation product patterns of the wyosine-akin tricyclic nucleoside 3a are in progress. At presence, despite the low yield of N-4 methylation leading to naturally occuring wyosine, the simplicity of the route provides this hypermodified nucleoside in pure form, in quantities allowing for much broader characterization than so far.

The approach bears also interesting similarity to the recent hypothesis concerning the sequence of events in the biogenetic formation of wyosine from guanosine /25/. It has been found, that lysine is involved in the biosynthesis of Y base in $tRNA^{Phe}$ of mammalian cells. On that basis it has been proposed that the first step of this biosynthesis is the formation of tricyclic base, by condensation between 2-amino group of guanosine and aldehyde group of α-amino-adipic acid semialdehyde, a metabolic of lysine. Methylation of the appropriate position completes the biosynthesis of Y base.

EXPERIMENTAL

General Methods

Melting points were determined in open capillaries on a Büchi SMP-20 apparatus and are uncorrected. Elemental analyses were performed on a Hewlett Packard 185 and a Perkin-Elmer 240 CHN analysers. Thin layer chromatography (TLC) was conducted on Merck precoated silica gel F_{254} plates (thickness 0.25 mm) using the following solvent systems measured by volume : A, chloroform-methanol (4:1); B, chloroform-methanol (9:1); C, dichloromethane-ethanol (9:1); D, dioxane-water (4:1); E, n-butanol-glacial acetic acid-water (5:3:2); F, isopropanol-concd.ammonia-water (7:1:2). For a preparative short column chromatography Merck TLC silica gel type 60 H was used. All evaporations were carried out in vacuo below 35°C.

The ultraviolet spectra were obtained on a Zeiss Specord UV-Vis spectrophotometer. The rate of glycosidic bond hydrolysis was measured on a Zeiss VSU-2P spectrophotometer using Vierordt method. The fluorescence spectra were recorded on a Hitachi MPF-4 fluorescence spectrophotometer at 20°C. The ^1H and ^{13}C NMR spectra were recorded on a JEOL FX 90 Q FT NMR spectrometer.

5,9-Dihydro-6-methyl-9-oxo-3-(β-D-ribofuranosyl)-5H-imidazo [1,2-a] purine (3a)

Guanosine (2, 20 g, 71 mmol) was reacted with sodium hydride and then with bromoacetone according to /2/. After the solution had been poured into 0.5 N KOH (1 L) and kept at room temperature for 4 h it was neutralized with Dowex exchange resin (50 W X 8, 50-100 mesh, pyridinium form). The resin was filtered off, the filtrate was concentrated to a small volume (250-300 mL). TLC in systems A and F showed unreacted guanosine (R_F 0.02 and 0.50) and a product of R_F 0.26 and 0.66 respectively. The solution was diluted with acetone (2-3 L), ethyl ether (400-600 mL) was added, thick, brown oil that separated out was dissolved in water and suspended on a column chromatography silica gel (15 g) by evaporation of the solvent. It was then applied on a silica gel short column (ø 7 cm, 200 g of adsorbent) and eluted with chloroform-methanol (4:1). Fractions containing the product were pooled, concentrated to approx. 0.5 L and left aside in a refrigerator; 3a crystallized gradually from this solution. Concentration of mother liquors afforded additional crop of pure crystals (17 g, 74% total) : mp 245°C dec (lit. /2/ 250°C dec).

Methylation of 3a with diazomethane. Reinvestigation of the reaction products.

Reaction was performed according to /2/. TLC in system A of the solution after methylation showed the presence of fluorescent product in addition to

unreacted substrate and 3b (R_F 0.25, 0.26, 0.29 respectively). After evaporation of the dimethylsulfoxide-methanolic solution TLC demonstrated that the fluorescent compound of R_F 0.25 had vanished and another of R_F 0.62 was formed. Dry residue was extracted with chloroform (2 x 10 mL), the extract was dried with sodium sulfate and evaporated to leave a flourescent material (1 mg , UV spectrum (H_2O, pH 6.8) of which was identical with that reported for wye (Y_t base) /2/.

5,9-Dihydro-6-methyl-9-oxo-3-(5-N,2'-O,3'-O,5'-O-tetra-acetyl-β-D-ribofuranosyl) imidazo [1,2-a] purine (3d).

A suspension of 3a (0.321 g, 1.0 mmol) in anhydrous pyridine (5 mL) and acetic anhydride (0.817 g, 8.0 mmol) was stirred magnetically at room temperature. After 1 h, when the suspension turned completely into the solution, TLC in solvent A showed no unreacted substrate, a small amount of 3c and a major spot of 3d (R_F 0.26, 0.77, 0.89 respectively). After additional 1 h stirring 3d was the only product. The solvents were removed in vacuo and the residue was coevaporated several times with isopropanol until a solid was obtained. It was dissolved in chloroform and chromatographed on a silica gel short column (ϕ 2.5 cm, 20 g of absorbent) with chloroform-methanol (95:5) as an eluent, to give TLC homogenous 3d (0.43 g, 88%). Analytical material was obtained by recrystallization from isopropanol: mp 67-68°C.

Anal. Calcd for $C_{21}H_{23}N_5O_9$: C,51.53; H,4.74; N,14.21.
Found: C,51.54; H,4.97; N,14.03.

5,9-Dihydro-6-methyl-9-oxo-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-5H-imidazo [1,2-a] purine (3c).

A suspension of 3a (3.21 g, 10 mmol) in anhydrous pyridine (50 mL) and acetic anhydride (8.17 g, 80 mmol) was reacted as described for 3d. The oily residue of 3d obtained after evaporation of solvents was treated with a mixture pyridine-methanol-water (1:1:1) at room temperature. TLC in solvent A showed complete transformation of 3d into 3c within 2 h. The solvents were then removed in vacuo and the residue was coevaporated several times with isopropanol until a solid was obtained. This material was dissolved in chloroform and chromatographed on a silica gel short column (ϕ 5.5 cm, 100 g of adsorbent) with chloroform-methanol (95:5) as an eluent to give analytically pure 3c (solid foam, 3.84 g, 86%).

Anal. Calcd for $C_{19}H_{21}N_5O_8$: C,51.01; H,4.73; N,15.65.
Found: C,50.70; H,4.67; N,14.95.

Methylation of 4-desmethylwyosine triacetate (3c) with diazomethane in dichloromethane.

3c (0.447 g, 1 mmol) in form of a solid foam was treated at room temperature with saturated at 0°C dichloromethane solution of diazomethane (30 mL, approx. 105 molar equivalents, according to titration with benzoic acid). After 10 min, TLC in system B showed two minor spots, intensively fluorescent under 360 nm UV lamp, one major product, and unreacted substrate (R_F 0.74, 0.67, 0.63 and 0.51 respectively). Prolongation of the reaction time did not result in the increase of the amount of fluorescent compounds, but in degradation. The unreacted methylating agent and the solvent were evaporated. The residue was dissolved in dichloromethane and chromatographed on a silica gel short column (ø 5.5 cm, 120 g of adsorbent) with dichloromethane-ethanol (975:25) as an eluent. Eluate was collected in 20 mL fractions monitored by TLC in system B, fractions were combined and evaporated. The composition of the fractions found from ^{1}H NMR spectra TABLE 4 and the amounts of material were as follows (in order of elution): I, unidentified fluorescent nucleoside acetate (9 mg): II, wyosine triacetate 1e (12.5 mg); III, 3e (380 mg); IV, 3c (50 mg).

4,9-Dihydro-4,6-dimethyl-9-oxo-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl) imidazo [1,2-a] purine (wyosine triacetate, 1e).

Fraction II of R_F 0.67 in system B and 0.79 in system C obtained from the above chromatographic separation was pure according to ^{1}H and ^{13}C NMR spectra. Detailed chromatographic and spectroscopic data are given in TABLES 2-4. Additional amount of 1e (1.5 mg) contained in fraction III was isolated when these fractions from several runs were combined, part of 3e removed by crystallization from isopropanol and remaining mother liquors chromatographed on a short column. Total yield 14 mg, 3.0% of solid foam.

5,9-Dihydro-5,6-dimethyl-9-oxo-3-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl) imidazo [1,2-a] purine (3e).

Fraction III (0.38 g, 82%) was recrystallized from isopropanol, mp 107-109°C.

Anal. Calcd for $C_{20}H_{23}N_5O_8$: C,52.06; H,5.02; N,15.18.
Found: C,52.00; H,5.38; N,14.74.

4,9-Dihydro-4,6-dimethyl-9-oxo-3-(β-D-ribofuranosyl) imidazo [1,2-a] purine (wyosine, 1a).

1e (10 mg) was dissolved in 14 N anhydrous methanolic ammonia (1 mL) and kept at room temperature. TLC in system A showed the reaction to be complete after 2.5 h. Wyosine, 1a that crystallized out from the reaction mixture was

collected by filtration (6 mg). Additional amount of nucleoside was isolated by evaporation of the mother liquors and recrystallization from methanol making total 6.5 mg, 90%; mp 231°C dec. Compound was pure according to ^{1}H and ^{13}C NMR spectra.

Detailed chromatographic and spectroscopic data are given in TABLES 2-4.

Hydrolysis of the glycosidic bond 1a. Determination of the rate.

Basing on the differences in the UV spectra of wyosine and wye base, Vierordt method was used. 1a (2 OD_{296}, 0.25 μmol) in 0.1 M citrate buffer (2 mL, pH 2.9) was thermostated at 37°C. Samples (200 μl) were withdrawn at 20-min time intervals, diluted with phosphate buffer (1 M, pH 7) to 2 mL volume and the UV absorbance was measured at 294 and 300 nm and at 294 and 312 nm. From the obtained values the amounts of the nucleoside and the base in time t were calculated; $t_{1/2}$ 55 min (found graphically).

5,9-Dihydro-5,6-dimethyl-9-oxo-3-(β-D-ribofuranosyl) imidazo [1,2-a] purine (3b).

3e (0.461 g, 1 mmol) was dissolved in 14 N anhydrous methanolic ammonia (20 mL) and kept at room temperature. The time of complete deacetylation and work-up procedure were the same as for 1e. White crystals (0.310 g, 93% total) in all respects identical with 3b obtained from the methylation reaction according to /2/.

1,9-Dihydro-1,6-dimethyl-9-oxo-3-(β-D-ribofuranosyl) imidazo [1,2-a] purine (4).

Method A. A solution of 3a (0.321 g, 1 mmol) in dimethylacetamide (10 mL) was treated with dimethyl sulfate (0.164 g, 1.3 mmol) and kept at room temperature until TLC in systems A, D and E showed the completion of the reaction. R_F-s of the substrate and of the methylation product were respectively: 0.26, 0.02 (A); 0.84, 0.33 (D); 0.58, 0.40 (E). The mixture was cooled to 10°C, carefully adjusted to pH 8-8.5 with concentrated aqueous ammonia and then diluted with acetone (100 mL) and ethyl ether (50 mL). The precipitate that isolated was collected by filtration and recrystallized from methanol to give an analytical sample of 4 (0.219 g, 65% : mp 165-166°C dec.

Anal. Calcd for $C_{14}H_{17}N_5O_5 \cdot {}^{1}/_{2}H_2O$: C,48.84; H,5.27; N.20.34

Found: C,48.41; H,5.00; N,20.09.

The amount of hydration was determined from ^{1}H NMR spectrum. All attempts to remove water, by heating in vacuo or by azeotroping, resulted in partial decomposition.

The reaction of 3a with methyl iodide under the same conditions needed approx. 30 h for completion. The attempts to transform the salt formed in

the reaction into mesoionic nucleoside were unsuccessful. Reaction did not take place at pH 8.5, higher values of pH resulted in imidazolium ring-opened product R_F 0.79 in system D, λ^{pH10}_{max} 234, 278 nm.

Method B. To a stirred solution of 7-methylguanosine 5 (0.297 g, 1 mmol) in dry dimethyformamide (5 mL) was added powdered potassium carbonate (0.152 g, 1.1 mmol) and bromoacetone (0.151 g, 1.1 mmol). The reaction was carried out at room temperature until TLC in system D showed the complete disappearance of the substrate (R_F 0.15, 1h). The mixture was neutralized with acetic acid, if needed, filtered through celite and the solvent was evaporated in vacuo. The residual solid was recrystallized from methanol to give analytically pure product in all respects identical with 4 obtained by method A.

Anal. Calcd for $C_{14}H_{17}N_5O_5 \cdot {}^1/_2H_2O$: C,48.84; H,5.27; N,20.34.
Found: C,48.93; H,5.14; N,20.30.

Ring-opening reaction of 4. Determination of the rate.

Half time for the ring opening was measured in 0.1 M Tris-HCl buffer, pH 9.85 and 25°C. Aqueous solution of 4 (1 mL) was combined with 0.2 M Tris HCl buffer (1 mL). At time t_0 this solution contained 1.4 OD_{234} of 4. The ultraviolet spectrum was recorded at 5-min time intervals. The increase in absorbance at 234 nm corresponded to the formation of the ring opened product. ΔA_{234} 0.56, t_∞ 90 min, $t_{1/2}$ 13 min.

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