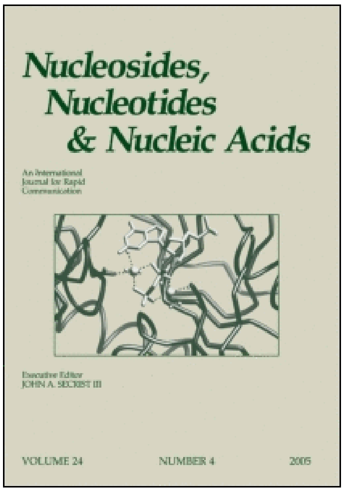

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# 1, $N^2$-Ethenoguanosine: Three Methods of Synthesis

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# 1,$N^2$-ETHENOGUANOSINE: THREE METHODS OF SYNTHESIS

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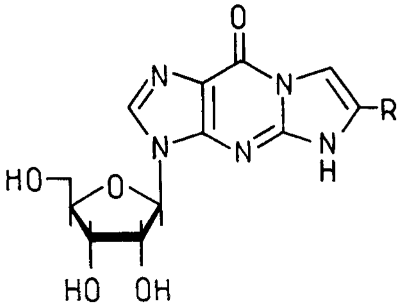
ABSTRACT: Three novel approaches to synthesis of 1,$N^2$-ethenoguanosine from guanosine: i) reaction with aq. chloroacetaldehyde at pH 9-10 ii) application of anhydrous chloro- and bromoacetaldehydes iii) 1-alkylation with bromoacetaldehyde diethyl acetal, subsequent hydrolysis to aldehyde on the nucleoside level and cyclization - afforded the title compound in satisfactory yields.

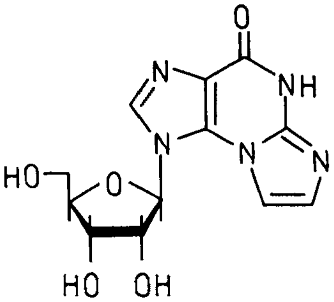
Among the four nucleic acid constituents, only adenosine and cytidine react readily with chloroacetaldehyde in aqueous solution[1-6] to form the etheno derivatives: 1,$N^6$-ethenoadenosine and 3,$N^4$-ethenocytidine, respectively. That interesting reaction has found many applications for the chemical modification of nucleic acids[7-9]. In turn, guanosine remains practically intact under conditions suitable for modification of adenosine and cytidine[5].

Under appropriate conditions, however, guanosine may form two different derivatives of the etheno type: the non-fluorescent 1,$N^2$-ethenoguanosine (1a; the "linear" form[10]); and the fluorescent $N^2$,3-ethenoguanosine (2; the "angular" form). The latter nucleoside, its 2'-deoxy derivative and the respective mono-,di- and triphosphates has been recently synthesized[11,12] in satisfactory yield in the reaction of $O^6$-benzylguanine system with bromoacetaldehyde, similarly to the previously reported synthesis of $N^2$,3-ethenoguanine[13]. Chemical synthesis of the linear 1,$N^2$-ethenoguanosine (1a)



1a: R=H

b: R=$CH_3$



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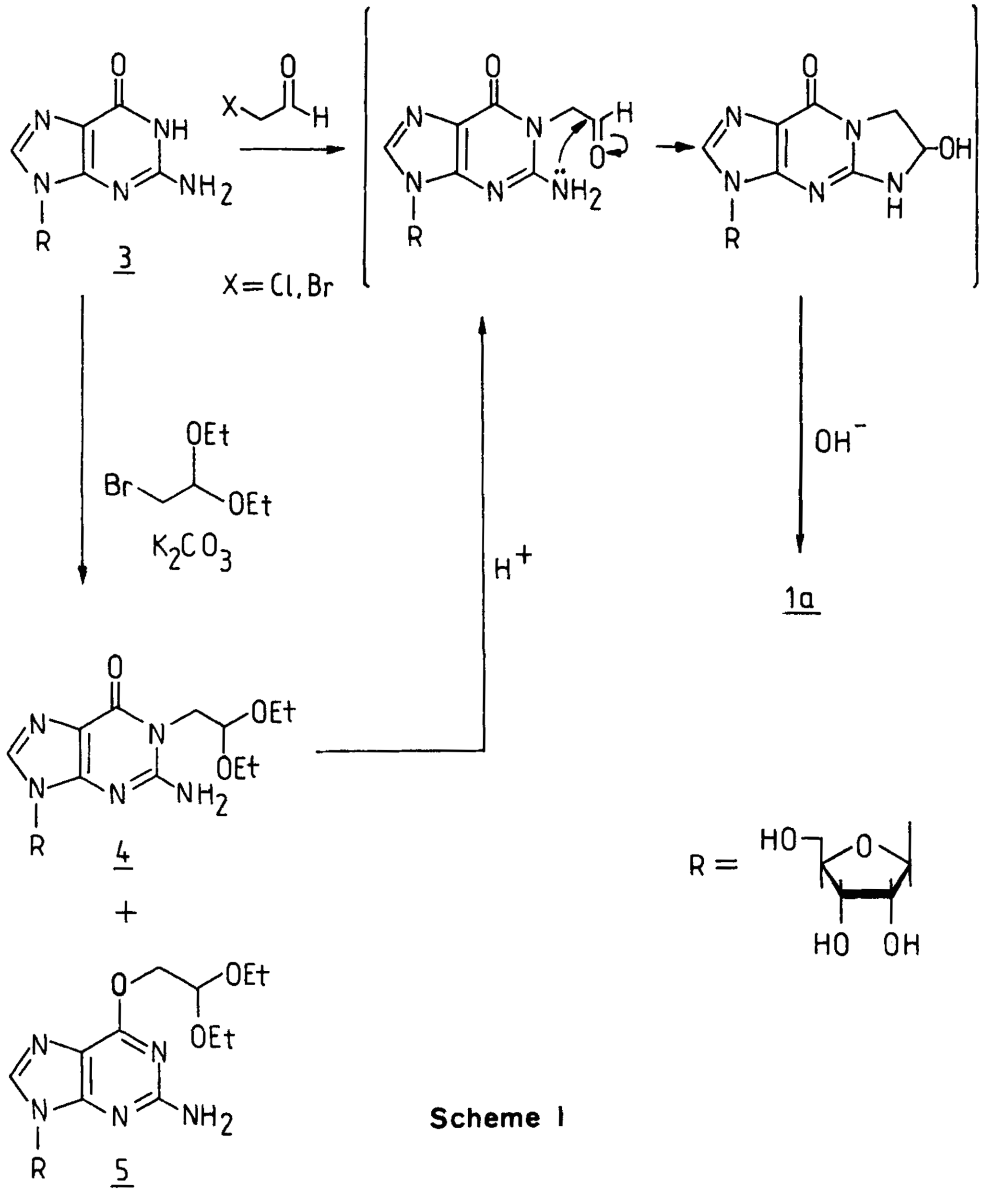
has been considerably less efficient so far. In 1977 Sattsangi et al.[13] reported the reaction of guanosine with aqueous chloroacetaldehyde under physiological conditions (pH 6.35-6.45), that after 3-7 days afforded 1a in the yield of 7.5%.

Since that time the linear tricyclic systems of guanosine have gained much attention due to the observation that $1,N^2$-isopropenoguanosine (1b; 4-desmethylwyosine)[14,16] and its anologues may serve as useful synthons for preparation of either $N^2$-alkyl[16] or 3-alkyl[17] derivatives of guanosine, that could not be obtained by its direct alkylation. Moreover, it has been recently shown that 9-[(2-hydroxyethoxy)-methyl]-$1,N^2$-isopropenoguanosine exhibits potent and selective antiherpetic activity *in vitro*[18]. Therefore, it would be also of interest to evaluate biological activities of $1,N^2$-ethenoguanosine and its acyclic analogues.

In this paper, I would like to report and compare three synthetic methods that may solve the problem of more efficient synthesis of the linear $1,N^2$-ethenoguanine system (Scheme I).

### Method A. Reaction of guanosine with aqueous chloroacetaldehydes at higher pH values.

As it was mentioned above, guanosine reacted very slowly with aqueous chloroacetaldehyde at physiological pH[13]. However, the results of more systematic study of the pH effect on the etheno derivative formation[5], performed in a limited range of pH values 3-7, might suggest that synthesis



**Scheme I**

of 1,N$^2$-ethenoguanosine would be more effective at higher pH values. Indeed, my introductory experiments carried out in slightly alkaline media confirmed that expectation. The optimum of pH for that reaction was found as 9-10. Thus, treatment of guanosine (3) with 50% aqueous chloroacetaldehyde in a mixture of dioxane - 2N sodium hydroxide (pH 9-10) gave after chromatographic purification 1,$N^2$-ethenoguanosine (1a) in 13% yield. A further increase of pH value (>10) did not facilitate the synthesis of 1a, but resulted in formation of an unstable, unidentified side-product of a higher $R_F$ value than that of 1a (0.29 and 0.23 in chloroform - methanol 4:1, respectively).

Method B. Reaction of guanosine with anhydrous haloacetaldehydes.

It has been previously reported, that reaction of 1-sodium derivative of guanosine with bromoacetone in dimethyl sulfoxide followed by treatment of the reaction mixture with 0.5 N potassium hydroxide gave 1,$N^2$-isopropenoguanosine (1b) in good yield[14]. Preparation of 1,$N^2$-etheno compounds in that way appeared to be considerably more difficult due to the well known instability of pure haloacetaldehydes. In the present work, the best results were obtained when chloro- or bromoacetaldehydes were used in freshly prepared ethereal solutions.

Reaction of the guanosine N-1-sodium salt in dimethyl sulfoxide with 90% chloroacetaldehyde in ethyl ether at 50°C for 16 h, and subsequent treatment of the reaction mixture with aqueous ammonia in order to dehydrate an intermediate product possessing $-CH_2CH(OH)-$ bridge between the 1-endo and $N^2$-exo nitrogen atoms (see Scheme I), afforded the etheno product 1a in 24% yield. In a similar experiment, application of 40% bromoacetaldehyde in ethyl ether instead of chloroacetaldehyde gave 1a in 25% yield.

Method C. Synthesis of 1,$N^2$-ethenoguanosine *via* 1-(2,2-diethoxyethyl) intermediate.

This simple and efficient method involves two preparative steps:

i) 1-alkylation of guanosine with bromoacetaldehyde diethyl acetal;

ii) acidic hydrolysis of the 1-substituted intermediate to an aldehyde on the nucleoside level, followed by spontaneous intramolecular cyclization and dehydration leading to 1,$N^2$-ethenoguanosine.

Reaction of guanosine (3) with an excess of bromoacetaldehyde - diethyl acetal (2-bromo-1,1-diethoxyethane) in dimethylformamide in the presence of solid potassium carbonate at 90°C for 3 days gave 1-(2,2-diethoxyethyl)guanosine (4) in the yield of 56% and an isomeric side-product, identified (UV, $^1H$ and $^{13}C$ NMR) as $O^6$-(2,2-diethoxyethyl)guanosine (5, 10%). Formation of the latter product was not surprising, since it had been shown that reaction of guanosine with alkyl iodides in the presence of potassium carbonate produced $O^6$-alkylguanosines in addition to the 1-alkylated products[19].

Alkylation of guanosine by bromoacetaldehyde diethyl acetal was also studied under different reaction conditions in order to improve its regiospecificity, but the yields of 4 were considerably lower than that of the experiment described above. For example, reaction of 1-sodium salt of guanosine (obtained by using sodium hydride) with the acetal in dimethyl sulfoxide gave 4 in 12% yield, and the $O^6$-alkylated product (5) was still present in the reaction mixture.

The acetal derivative 4 was then hydrolyzed in 1 N hydrochloric acid for 24 h, what gave quantitatively the desired product 1a (90% yield after purification).

Structures of the products were confirmed by the proton and carbon-13 magnetic resonance spectroscopy, ultraviolet spectra and elemental analyses. The UV and $^1H$ NMR spectra of $1,N^2$-ethenoguanosine (1a) are in good agreement with the previously reported data[13]. The $^{13}C$ NMR spectrum closely resembles that of $1,N^2$-isopropenoguanosine (1b)[20], but the difference between $1,N^2$-isopropeno and $1,N^2$-etheno systems can be clearly seen. In particular, the signal of =CHNH etheno carbon of 1a is shifted upfield to 116.49 ppm in comparison to the value 126.18 ppm for 1b. The $^{13}C$ NMR method may be also useful in distinguishing between 1-(2,2-diethoxyethyl)guanosine (4) and its $O^6$-substituted isomer (5). Their $^{13}C$ NMR spectra are very similar to those reported[21] for 1-ethyl- and $O^6$-ethylguanosines, respectively. The signal of methylene carbon 1-$CH_2$- (compound 4) appears at

44.70 ppm, when the signal of $O^6$-$CH_2$- (compound 5) is shifted downfield to 65.23 ppm.

## Conclusion

Although Methods A and B offer higher yields of 1,$N^2$-ethenoguanosine than that reported[13], it does not seem likely that haloacetaldehydes would be of general importance in an efficient synthesis of the 1,$N^2$-ethenoguanine system. The presented here two-step procedure (Method C) gives the linear ethenoguanosine in a much better yield (overall yield of transformation 3 to 1a 50.4%), avoiding a laborious preparation of highly toxic haloacetaldehydes and affording the product of higher purity (see melting points) than those obtained in Methods A and B.

Studies on biological activity of 1,N-2-ethenoguanosine and its acyclic analogues[22] are in progress.

## EXPERIMENTAL

Melting points were determined on a micromelting point apparatus in open capillaries and are uncorrected. The ultraviolet spectra were measured in water on a Zeiss Specord UV-Vis spectrophotometer. The $^1H$ and $^{13}C$ NMR spectra were recorded with a Jeol FX 90Q FT NMR spectrometer in $d_6$-DMSO at 89.55 and 22.50 MHz, respectively. Chemical shifts are expressed in $\delta$ values (parts per million) relative to $Me_4Si$ as an internal standard. Thin-layer chromatography was conducted on Merck precoated silica gel $F_{254}$ Type 60 plates and $R_F$ values are given for chloroform - methanol (4:1, v/v). For a preparative short-column chromatography Merck TLC gel $HF_{254}$ Type 60 was used. Elemental analyses were performed on a Perkin Elmer 240 Elemental Analyzer and the presence of water in analytical samples was confirmed by the $^1H$ NMR.

Bromoacetaldehyde diethyl acetal and 50-55% aq. chloroacetaldehyde were products of Fluka AG.

### Preparation of chloroacetaldehyde solution in ethyl ether

A commercial 50-55% aqueous solution of chloroacetaldehyde (5 mL) was extracted with diethyl ether (20 mL). The organic layer was separated, dried with $Mg_2SO_4$ and concentrated under diminished pressure without heating to a volume of ca 2.5 mL. The obtained colorless liquid was dried over molecular sieves 4A and was directly used in the reaction with guanosine. According to the $^1H$ NMR assay the solution contained ca 90% of chloroacetaldehyde: 4.07 (d,2), 9.61 (t,1), J = 1.7 Hz.

Preparation of ethereal solution of bromoacetaldehyde

A diluted ethereal solution of bromoacetaldehyde was obtained either by direct bromination of acetaldehyde[23] or by hydrolysis of bromoacetaldehyde diethyl acetal in 20% $H_2SO_4$ under reflux for 25 min, followed by extraction with diethyl ether. The application of 1 N hydrochloric acid[11] instead of sulfuric acid for the latter reaction resulted in a mixture of bromo- and chloroacetaldehydes in a ratio 1:1, approximately, as determined by the $^1H$ NMR.

The ethereal solution was then dried with $MgSO_4$, and concentrated in vacuo to a final concentration 40-60% as assayed by the $^1H$ NMR: 3.86 (d,2), 9.54 (t,1), J = 2.4 Hz. This preparation could be stored over molecular sieves 4A at -10°C for several days without decomposition. More concentrated samples underwent a fast decomposition.

1-(2,2-Diethoxyethyl)guanosine (4)

Well powdered potassium carbonate (3.66 g, 26.47 mmol) was added to stirred suspension of guanosine (5.0 g, 17.65 mmol) in DMF at 90°C, followed by bromoacetaldehyde diethyl acetal (4.12 mL; 5.22 g, 26.47 mmol) after 5 min, when guanosine was completely dissolved. Stirring was continued at this temperature for 3 days. Next portions of potassium carbonate (2 x 1.22 g, 8.82 mmol) and the acetal (2 x 1.37 mL; 1.74 g, 8.82 mmol) were added after 24 and 48 h of the reaction. The reaction mixture was then filtered through a layer of Celite and the insoluble material was washed with DMF. The filtrate was evaporated to dryness, and a resulting brown oil was dissolved in 40% aq. ethanol, then adsorbed on silica gel (70-230 mesh, 50 g) by evaporation. The dried gel was then applied on a silica gel column (5.5 x 12 cm) and products were eluted in a chloroform - methanol gradient (from 95:5 to 85:15, respectively).

Fractions # 64-72 (@ 20 mL) containing the $O^6$-substituted product 5 were evaporated to give 699 mg (10%) of a solid foam. Attempted crystallization from alcohols and water failed. An analytical sample of 5 was obtained by precipitation with ethyl ether from its solution in chloroform. $R_F$ 0.66. Anal. Calcd for $C_{16}H_{25}N_5O_7 \cdot 0.5\ H_2O$ (408.41) %C, 47.06; %H, 6.42; %N, 17.15. Found: %C, 47.46; %H, 6.44; %N, 16.94. $\lambda_{max}$ ($H_2O$) 248 nm ($\varepsilon$ = 9,300), 281 (9,700). $^1H$ NMR 1.14 (t,6,$CH_3$), 3.30-3.78 (m,6,$OCH_2$ and 5'H), 3.90 (q,1,4'H) 4.13 (q,1,3'H), 4.43 (m,3,$C\underline{H}_2CH$ and 2'H), 4.88 (t,1,OCHO), 5.09 (m,2,3'- and 5'-OH), 5.38 (d,1,2'-OH), 5.80 (d,J=5.9 Hz,1,1'H), 6.43 (br,2,$NH_2$), 8.11 (s,1,8-H). $^{13}C$ NMR 15.17 ($CH_3$), 61.39 (C-5'), 61.82 ($O\underline{C}H_2CH_3$), 65.23 ($O^6CH_2$), 70.33 (C-3'), 73.41 (C-2'), 85.22 (C-4'), 86.69 (C-1'), 99.37 (OCHO), 113.96 (C-5), 138.05 (C-8), 154.34 (C-4), 159.51 (C-2), 159.88 (C-6).

Evaporation of fractions # 75-103 afforded the homogeneous by tlc main product (4) as a white solid foam. Yield 3.94 g (56%). Attempted crystallization from water, alcohols, benzene, ethyl acetate failed. An analytical sample of the compound was obtained by precipitation with ethyl ether from its solution in chloroform. $R_F$ 0.59. Anal. Calcd for $C_{16}H_{25}N_5O_7 \cdot H_2O$ (417.42) %C, 46.03; %H, 6.52; %N 16.78. Found: %C 46.35; %H 6.60; %N, 16.39. $\lambda_{max}$($H_2O$) 254 nm ($\varepsilon$ = 12,300), 271 (9,500, sh). $^1H$ NMR 1.08 (t,6, $CH_3$), 3.30-3.79 (m,6,$OCH_2$ and 5'H), 3.87 (q,1,4'H), 4.08 (m,3,$NCH_2$ and 3'H), 4.41 (q,1,2'H), 4.72 (t,1,CH), 4.98 (t,1,5'-OH), 5.09 (d,1, 3'-OH), 5.37 (d,1,2'-OH), 5.70 (d,J=6.3 Hz,1,1'H), 6.81 (br, 2,$NH_2$), 7.96 (s,1,8-H). $^{13}C$ NMR 15.12 ($CH_3$), 44.70 ($NCH_2$), 61.44 (C-5'), 62.85 ($OCH_2$), 70.38 (C-3'), 73.69 (C-2'), 85.22 (C-4'), 86.20 (C-1'), 100.34 (OCHO), 116.06 (C-5), 135.89 (C-8), 149.38 (C-4), 154.52 (C-2), 156.58 (C-6).

### 1,$N^2$-Ethenoguanosine (1a)

Method A. A solution of 2 N sodium hydroxide (0.625 mL) was added to a suspension of guanosine (566.5 mg, 2 mmol) in dioxane (5 mL). A clear solution obtained after 2 min of stirring was then treated with 50% aq. chloroacetaldehyde in

20 portions (total 0.4 mL, ca 3 mmol) during 2 h. The pH value was maintained at 9-10 by addition of 2 N NaOH. Stirring was continued for the next 10 h. The reaction mixture was then further alkalized with conc. $NH_4OH$ (10 mL). After 1 h at room temperature an excess of ammonia was gently evaporated *in vacuo* and the reaction mixture was adsorbed on silica gel (70-230 mesh, ca 8 g) by evaporation. The gel was applied on a silica gel short column (3.5 x 10 cm) and the product was eluted in a gradient of chloroform - methanol (9:1 to 4:1), 15-mL fractions. Fractions # 23-41 containing chromatographically pure 1,N$^2$-ethenoguanosine (1a) were evaporated to give a white solid. Crystallization of 1a from water gave 80 mg (13%) of crystalline material, m.p. 249-253° (dec) (Ref.[13] 252-253° dec).The ultraviolet and $^1$H NMR spectra were identical with those described in Method C.

Method B. Sodium hydride (26.4 mg, 1.1 mmol) in 60% suspension in oil was added to an anhydrous solution of guanosine (283.3 mg, 1.0 mmol) in dimethyl sulfoxide (5 mL) and this mixture was stirred with exclusion of moisture for 40 min. The clear solution was treated with a 90% solution of chloroacetaldehyde in ether (178 µL, ca 2.2 mmol), and the reaction mixture was stirred at 50°C for 16 h, then it was made basic by addition of conc. $NH_4OH$ (1 mL). After 15 min the solution was concentrated *in vacuo* to ca 2 mL, and diluted with acetone (25 mL) and ethyl ether (15 mL), then maintained at 0°C for 3 h. The resulted oily precipitate was separated by decantation, washed with ether and dried under reduced pressure. The residue was dissolved in water, adsorbed on silica gel (5 g), and the product 1a was isolated by short-column chromatography as described in Method A, what yielded 74 mg (24%) of 1a as a white solid. Crystallization from methanol gave a compound with m.p. 248° (dec); the UV and NMR data like in Method C.

In a similar experiment, the ethereal solution of chloroacetaldehyde was replaced by 40% bromoacetaldehyde in ether. Reaction was carried out at room temperature for 2 h, then at 45°C for 30 min. Yield 25%.

Method C. 1-(2,2-Dietoxyethyl)guanosine (4; 1.0 g, 2.50 mmol) was dissolved in 1 N hydrochloric acid (20 mL) and left at room temperature for 24 h. The solution was then alkalized by addition of conc. $NH_4OH$ (8 mL). Thin-layer chromatography showed a quantitative conversion to 1a. The reaction mixture was adsorbed on silica gel as it was described in the Method A. The dried gel was applied on a silica gel short column (5 x 6 cm) and the product was eluted with chloroform - methanol - triethylamine 85:14:1 (measured by volume). Fractions # 26-55 (@ 20 mL) containing the homogeneous by tlc 1,$N^2$-ethenoguanosine (1a) were evaporated to give 695 mg (90%) of a white solid. This material was then dissolved in aq. ammonia. The solution was concentrated under diminished pressure until first crystals appeared, then stored at 0°C overnight. The crystalline material was collected by filtration, washed with a small amount of cold ethanol and dried in a vacuum desiccator. m.p. 256-257.5° (dec). $R_F$ 0.23. Anal. Calcd for $C_{12}H_{13}N_5O_5$ (307.27) %C, 46.91; %H, 4.26; %N, 22.79. Found: %C, 46.69; %H, 4.19; %N, %N, 22.91. $\lambda_{max}(H_2O)$ 227 nm ($\varepsilon$=34,600), 285 (12,000). $^1$H NMR 3.63 (m,2,5'H), 3.93 (q,1,4'H), 4.16 (q,1,3'H), 4.49 (q,1, 2'H), 5.11 (m,2, 3'- and 5'-OH), 5.40 (d,1,2'-OH), 5.84 (d, J=5.6 Hz,1,1'H), 7.43 and 7.62 (2d, J=2.6 Hz,2,CH=CH), 8.15 (s,1,8-H), 12.32 (br,1,NH). $^{13}$C NMR 61.50 (C-5'), 70.44 (C-3'), 73.80 (C-2'), 85.33 (C-4'), 87.23 (C-1'), 106.90 (>NCH=), 115.51 (C-5), 116.49 (-HNCH=), 137.35 (C-8), 145.91 (C-2), 150.35 (C-4), 151.38 (C-6).

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