1,N^2-Ethenoguanine and N^2,3-Ethenoguanine. Synthesis and Comparison of the Electronic Spectral Properties of These Linear and Angular Triheterocycles Related to the Y Bases

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We have shown that the reaction of guanosine with chloroacetaldehyde in aqueous solution in the physiological pH range yields 1,N^2-ethenoguanosine (5,9-dihydro-9-oxo-3-β-D-ribofuranosylimidazo[1,2-a]purine). This compound could be hydrolyzed to 1,N^2-ethenoguanine (5,9-dihydro-9-oxoimidazo[1,2-a]purine), which was also prepared authentically by hydriodic acid treatment of the glyoxal–guanine adduct. The 1,N^2-ethenoguanine, which is an unsubstituted (at N-4, C-6, and C-7) Y-type base, is not fluorescent under the same conditions at which the 4-methyl compounds fluoresce. By contrast, the isomeric and angular N^2,3-ethenoguanine (8,9-dihydro-9-oxoimidazo[2,1-b]purine) is fluorescent ($\lambda_{excitation}$ 262 nm, $\lambda_{emission}$ 410 nm). The N^2,3-ethenoguanine synthesis was initiated by the reaction of chloroacetaldehyde with O^6-benzylguanine, O^6-methylguanine, and 2-amino-6-benzylthiopurine, followed by hydrogenolysis or hydrolysis, hydrolysis, and oxidation and hydrolysis, respectively. The reaction of guanosine is indicative of the damage that can result from the action of the mutagen chloroacetaldehyde on guanosine derivatives under physiological pH conditions.

The reaction of chloroacetaldehyde in aqueous solution with adenine- and cytosine-containing compounds[1,2] to produce 1,N^6- and 3,N^4-etheno-bridged compounds, respectively, has found wide application.[3,4] Interest stems from the biological activity generally evident at the nucleoside, nucleotide, and coenzyme level and from the species responsible for the fluorescence emission properties.[5,6] The crystal and molecular structures of suitable derivatives have been determined.[7,8] We agree with Kochetkov, Shibaev, and Kost[1] that in the pH range most favorable for reaction at 37 °C of chloroacetaldehyde with adenosine (pH 4.5) and cytidine (pH 3.5), guanosine is not reactive.[2] When chloroacetaldehyde was used in this laboratory to modify tRNA in aqueous solution at different selected pHs,[9] guanosine as well as cytidine and adenosine residues appeared to be undergoing attack at pH 6.3,[10] within the optimum range for retention of tRNA tertiary structure.[11]

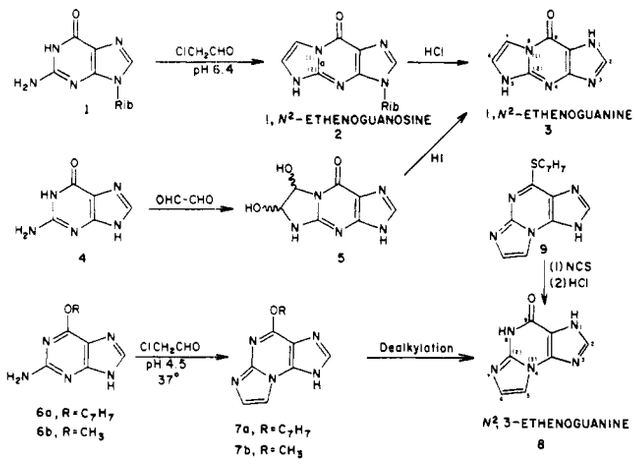
Since chloroacetaldehyde is known to be mutagenic[12,13] and is one of the likely liver metabolites of vinyl chloride, its reaction with guanosine under physiological conditions was of particular interest. Moreover, the possible development of fluorescence due to the formation of an additional ring suggested the value of product comparison with the fluorescent natural nucleosides Y[14-27] (wybutosine, Y-Wyo)[28] and Yt[29-31] (wyosine, Wyo)[28] and corresponding bases related to guanine.[32]

The reaction of guanosine (1) with chloroacetaldehyde in aqueous solution at 37 °C was followed by the development of ultraviolet absorption at 305 nm over a period of hours and over a pH range from 6.5 to 4.5. At pH 6.5 the reaction rate is significant, but is still less than one-third that of adenosine with chloroacetaldehyde under the same conditions. The relative reaction rate for the guanosine reaction falls off sharply with decreasing pH and is practically negligible at pH 4.5. A preparative reaction was run at carefully controlled pH (6.40 ± 0.05), monitored by comparison of the ultraviolet absorption intensities at λ_{max} 272 and 254 nm, and, for the optimum conversion, halted when these became equal. 1,N^2-Ethenoguanosine (2) was isolated from the reaction mixture by chromatography on cellulose followed by high performance liquid chromatography on cation exchange resin. The elemental analysis and the mass spectrum were consistent with the introduction of an etheno bridge, which was also indicated by the pair of doublets (6-H or 7-H, δ 7.43 or 7.62, J_{67} = 2.5 Hz, along with 2-H, δ 8.16) for the etheno protons in the NMR spectrum. The linear (1,N^2-etheno) (2) rather than angular (N^2,3-etheno) tricyclic structure was assigned to the guanosine–chloroacetaldehyde product, inter alia, by the close similarity of its ultraviolet spectra (Figure 1) with those of 5,9-dihydro-6-methyl-9-oxo-3-β-D-ribofuranosyl-5H-imidazo[1,2-a]purine (2, with CH_3 at C-6)[31] in acidic, neutral, and basic media. The 5-H (2) rather than the 4-H tautomeric form was indicated by the close similarity of the ultraviolet absorption spectra, in acidic and neutral media, of the product with those of 5,9-dihydro-5,6-dimethyl-9-oxo-3-β-D-ribofuranosylimidazo[1,2-a]purine (2, with CH_3 at N-5 and C-6),[31] locked in the "5-H" form by the methyl substituent. The chemical shift of δ 7.43 in the product 2 could be assigned to the 7-H not only by comparison with the δ 7.36 and 7.43 values for the 7-H in the 6-methyl and 5,6-dimethyl models, respectively,[31] but, positively, by the observed reduction in intensity of the δ 7.43 signal relative to that of δ 7.62 when α-deuterio-enriched chloroacetaldehyde, e.g., $ClCD_2CHO$, was used in the reaction with guanosine. This method of NMR assignment based upon deuterium labeling had proved effective for the adenosine and cytidine reaction products with chloroacetaldehyde.[6-8,33]

The deribosidation of 1,N^2-ethenoguanosine (2) was effected by acid hydrolysis to produce 1,N^2-ethenoguanine (3). Guanine (4) itself did not react with chloroacetaldehyde under the conditions specified for the conversion of guanosine to the etheno-bridged product. However, compound 3 could be obtained unequivocally by treating the adduct formed from guanine and glyoxal (5, the tautomeric form is written conventionally)[34] with 47% hydriodic acid at 55–60 °C for 5–6 days. The elemental analysis and the mass spectrum were satisfactory for the indicated product 3. NMR assignments for the hydriodide of 3 are comparable with those for compound 2 except for the signal for 2-H, which experienced a downfield shift of δ 1.2, suggestive of N-1/N-3 as the site of protonation. A similar downfield shift of δ 1.3 was observed for the signal of the corresponding 8-H upon conversion of guanosine to 7-methylguanosine.[35,36] Preference for the tautomeric form (1-H, 5-H) shown in 3 is adduced from comparison of the ultraviolet absorption spectra in acidic, neutral, and basic media (Figure 1) with those of models,[31] but the representation is not intended to be exclusive. All of the assembled data clearly support the structure assignment of the base as 1,N^2-ethenoguanine (5,9-dihydro-9-oxoimidazo[1,2-a]purine) (3) and the riboside as 1,N^2-ethenoguanosine (5,9-dihydro-9-oxo-3-β-D-ribofuranosylimidazo[1,2-a]purine) (2).

Despite structural similarities to the fluorescent Y bases and nucleosides, 1,N^2-ethenoguanine (**3**) is only weakly fluorescent and 1,N^2-ethenoguanosine (**2**) is nonfluorescent. The major structural difference is the fixed N-4 substitution in the natural Y series vs. the mobile 5-H in our synthetic series. We therefore decided to synthesize N^2,3-ethenoguanine, which contains the etheno bridge in an angular tricyclic system, for comparison of its spectroscopic properties—especially possible fluorescence emission—with those of its isomer **3**.

In order to increase the basicity (nucleophilicity) of the guanine ring system and to hinder sterically the reaction of chloroacetaldehyde at N-1, we selected O^6-benzylguanine (**6a**)[37] as the precursor of the angular system. The reaction of **6a** with chloroacetaldehyde in dilute solution proceeded smoothly at 37 °C and at pH 4.5, the optimal pH for adenosine. The O^6-benzyl-N^2,3-ethenoguanine (9-benzyloxyimidazo[2,1-*b*]purine) (**7a**) thus obtained was readily converted to N^2,3-ethenoguanine (8,9-dihydro-9-oxoimidazo[2,1-*b*]-purine) (**8**) by hydrolysis with 2 M hydrochloric acid or by hydrogenolysis over palladized charcoal.[38] Similarly, N^2,3-etheno-O^6-methylguanine (**7b**) was prepared from O^6-methylguanine[39] and was hydrolyzed by heating with concentrated hydrochloric acid on a steam bath to produce N^2,3-ethenoguanine (**8**). Consideration of the versatility of thioalkyl groups for subsequent transformations led us to prepare 6-benzylthio-N^2,3-ethenoguanine (**9**) from 2-amino-6-benzylthiopurine,[40,41] again with chloroacetaldehyde in dilute solution at 37 °C and pH controlled at 4.0–4.5. The conversion of **9** to **8** was accomplished by oxidation with *N*-



chlorosuccinimide followed by hydrolysis with hydrochloric acid.

The NMR spectra of **7a, 7b,** and **9** are characterized in the aromatic region by the presence of a singlet for 2-H and a pair of doublets for the etheno protons 5-H and 6-H. The latter signals are separated by about 0.4 ppm. The three protons, 2-H, 5-H, and 6-H, respectively, exhibit similar chemical shifts in **7a, 7b,** and **9**. The NMR spectra of the hydrochloride salts of **7a** and **7b** were almost identical for the 2, 5, and 6 protons. The spectra of the hydrochlorides prepared from **6a** and **6b** by reaction with $ClCD_2CHO$ lacked the signal at δ 8.38 for the 5-H, and the signal at δ 8.08 for the 6-H collapsed to a singlet with formal replacement of 5-H by 5-D. These assignments were carried over to the free bases (**7a,b**). We observed downfield shifts of 0.4–0.7 ppm for the 2-, 5-, and 6-H's and an increase in the J_{56} value from 1.5 to 2.6 for the etheno protons when **7a** and **7b** were converted to their hydrochlorides. The 6 proton was shifted maximally, which suggests that N-7 is the site of protonation in these compounds. Comparable shifts had been observed in the NMR spectrum of 3,N^4-ethenocytidine hydrochloride compared with that of the free

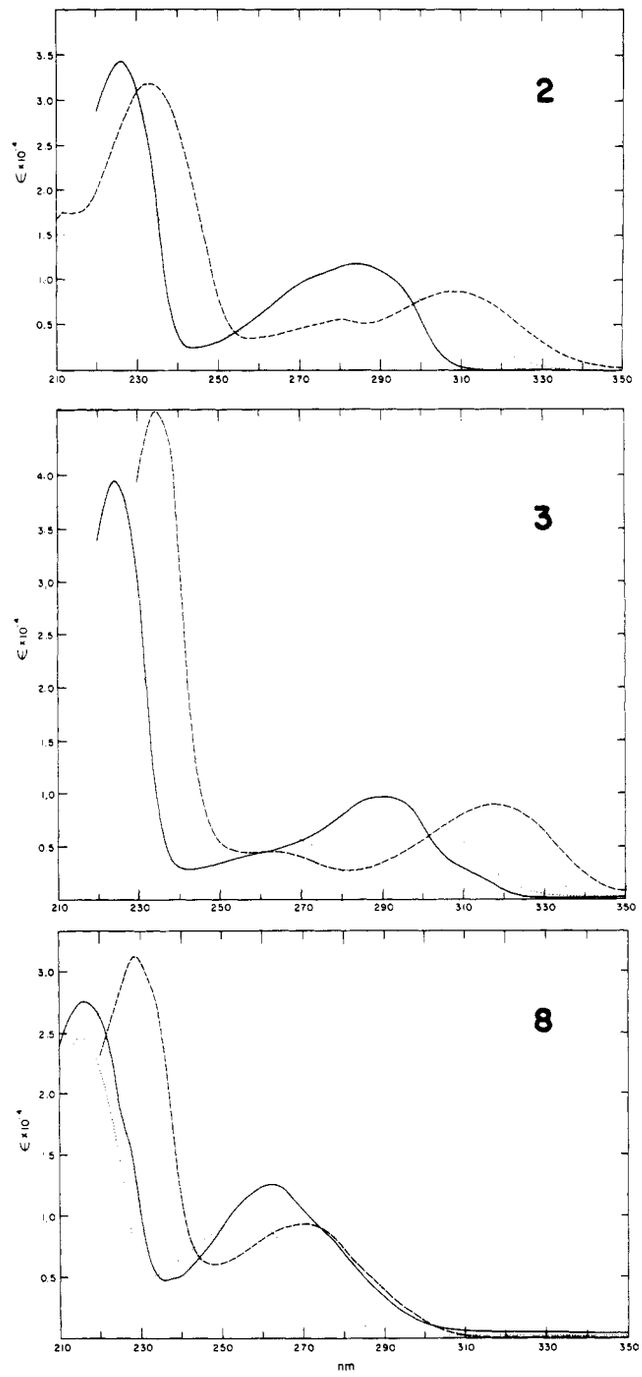


Figure 1. The UV spectra of 1,N^2-ethenoguanosine (**2**), 1,N^2-ethenoguanine (**3**), and N^2,3-ethenoguanine (**8**) in aqueous 0.05 M phosphate buffer, pH 7 (—), 0.1 N HCl (· · ·), and 0.1 N NaOH (- - -).

base.[6] The NMR spectrum of the angular ethenoguanine, 8,9-dihydro-9-oxoimidazo[2,1-*b*]purine (**8**), in the aromatic region showed parallel chemical shifts, that is, increasing δ following the order 6-H, 5-H, and 2-H (see Experimental Section), which were assigned conveniently by conversion of the 5-D precursors to the 5-deuterio-**8**.

Tricyclic aromatic and heteroaromatic systems of the linear type generally exhibit lower energy electronic transitions than their angular isomers.[42,43] This is true also for 1,N^2-ethenoguanosine (**2**) and 1,N^2-ethenoguanine (**3**) compared with N^2,3-ethenoguanine (**8**). The low-energy bands in the ultraviolet spectra (Figure 1) of the angular isomer **8** are shifted 30–50 nm toward shorter wavelength from those of the linear pair (**2, 3**). What is most impressive is the finding that the

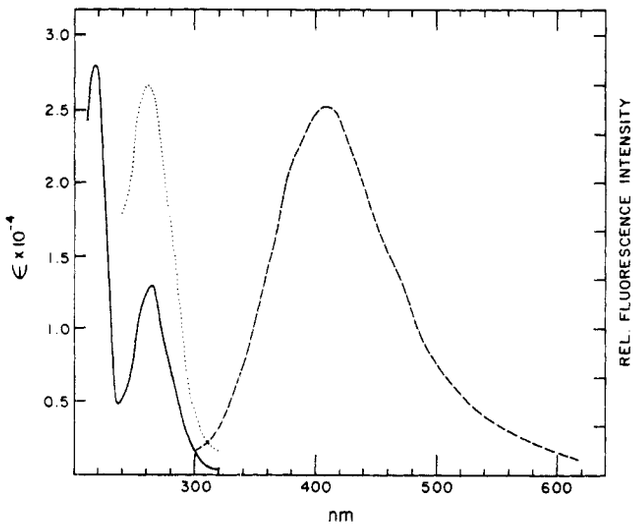


Figure 2. Ultraviolet absorption (—), corrected fluorescence excitation (···), and corrected fluorescence emission (- - -) spectra of N^2,3-ethenoguanine (8) in water, pH 6.8.

N^2,3-ethenoguanine (8) is *fluorescent*, whereas the linear 1,N^2-ethenoguanosine (2) is not. The fluorescence emission maximum is observed at 410 nm when compound 8 is irradiated near 280 nm.

A study of the fluorescence behavior of N^2,3-ethenoguanine (8) in solvents of decreasing polarity (water, ethanol, dioxane) showed the corrected excitation maxima to be consistent with the corresponding UV maxima (Table I, Figure 2). Although the wavelength of the emission maxima did not change significantly with decreasing solvent polarity, the relative quantum yields and fluorescence lifetimes showed substantial increases in going from water to ethanol to dioxane. Similar changes in emission characteristics have been observed with other fluorescent guanine derivatives.[44] Evleth and Lerner[45] indicate that theoretical analysis reasonably rationalizes the photophysical properties of 1,N^6-ethenoadenine (ϵ-adenine) by demonstrating a relationship with the highly fluorescent indolizines, whereas the relationship between a Y base and the indolizines is much less well characterized and only a vague relationship is found to exist. Neither 1,N^2-ethenoguanine (3) (5-H tautomer as opposed to the 4-CH_3 substitution in a Y base) nor N^2,3-ethenoguanine (2) has been subjected to analogous theoretical treatment as yet.

In conclusion, we have achieved a better understanding of the slow reaction of chloroacetaldehyde with guanosine under physiological conditions (pH ca. 6.4) and have established the structure of the major product formed in the reaction. This pH may be selected for the reaction of chloroacetaldehyde with more complex molecules containing guanosine. Where both guanosine and adenosine units are present and stereochemically available, both can react at pH 6.4. We have also produced a fluorescent derivative of guanine, namely N^2,3-ethenoguanine (8), that should possess, in protonated form, hydrogen-bonding characteristics like guanine and, as the base, hydrogen-bonding characteristics like a 3-substituted xanthine.

Experimental Section

Melting points were recorded on a Büchi melting point apparatus and are uncorrected. The NMR spectra were recorded by Mr. Steven Silber on a Varian Associates HA-100 spectrometer using tetramethylsilane as an internal standard. The ultraviolet spectra were obtained on a Beckman Acta MVI spectrophotometer. Corrected fluorescence emission and fluorescence excitation spectra were acquired on a Spex Fluorolog spectrofluorometer. All fluorescence measurements were made at room temperature (22 °C). The fluorescence measured for each solution was normalized for differences among the samples in optical density at the exciting wavelength and is therefore a quantitative representation of quantum efficiencies relative to quinine taken as 0.7.[46]

Table I. Fluorescence Emission and Excitation Data for 8

Solvent	Emission[a] λ_{max}, nm	Excitation[b] λ, nm	τ,[c] ns	Φ rel[d]
H_2O (pH 6.8)	410	262 (UV 262)	1.38 ± 0.01	0.03
EtOH	400	263 (UV 263)	2.23 ± 0.02	0.12
Dioxane	400	270 (UV 265)	2.73 ± 0.01	0.26

[a] Fluorescence emission spectra were measured with excitation at 280 nm, and corrected curves were obtained using correction factors supplied with the Fluorolog. [b] Corrected excitation spectra were recorded directly by holding emission at 415 nm. [c] Fluorescence lifetimes were measured by phase only. [d] Determined using corrected emission spectra by comparison with quinine sulfate (in 0.1 N H_2SO_4), which has a quantum yield of 0.7.[45]

Quantum efficiencies were obtained by using corrected spectral areas and also by means of a spectrofluorometer described by Weber et al.[47] The values obtained by the two methods were comparable. Fluorescence lifetimes were measured by Dr. David Jameson on a Model SLM subnanosecond spectrofluorometer. Thin-layer chromatography was carried out on Eastman chromagram sheets of cellulose or silica gel, with or without fluorescent indicator. After development of the chromatograms, spots were located with the aid of an ultraviolet lamp. The solvent systems employed were: solvent 1, 1-butanol–water (86:14); solvent 2, 2-propanol–water (7:3); solvent 3, ethyl acetate–1-propanol–2-propanol–water (4:2:1:2); solvent 4, water; solvent 5, chloroform–ethanol (80:20); solvent 6, chloroform–ethanol (70:30).

Preparative high performance liquid chromatography (HPLC) was done using a Chromatronix pump and UV detector and a Hewlett-Packard recorder equipped with a unit to provide automatic zero suppression. Glass columns designed by Drs. L. Kirkegaard and D. Cole, University of Illinois, were packed with suitable resins for the separations involved. Microanalyses were performed by Mr. Joseph Nemeth and associates, who also weighed samples for quantitative electronic absorption spectra. Low-resolution mass spectra were obtained by Mr. J. Wrona on a Varian-Mat CH-5 spectrometer coupled with a 6201 computer and STATOS recorder.

pH Profile of the Reaction of Chloroacetaldehyde with Adenosine and Guanosine. A solution of 35 mg (0.125 mmol) of guanosine in 35 mL of 0.5 M sodium acetate buffer (for pH 4.5 and 5.5) or potassium phosphate buffer (for pH 6.4) and 1 mL of 2 M aqueous chloroacetaldehyde solution was stirred in a stoppered flask at 37 °C. A similar arrangement was used for adenosine, with controls for buffer and chloroacetaldehyde and buffer blanks. Aliquots (1 mL) were taken at constant intervals, made up to 50 mL with 0.01 M NaOH, and the spectra were recorded. Absorption values corresponding to the formation of 1,N^6-ethenoadenosine (290 nm, ϵ 3700) and 1,N^2-ethenoguanosine (305 nm, ϵ 8600)[48] were corrected for buffer and chloroacetaldehyde blank to give A_{obsd}. Percentage of products formed ($A_{obsd}/A_{prod} \times 100$) was plotted against time in hours to obtain a semiquantitative comparison of reaction rates.

All chloroacetaldehyde reactions must be run in a well-ventilated hood, employing adequate precaution against exposure to any part of the body.[12,13]

1,N^2-Ethenoguanosine (5,9-Dihydro-9-oxo-3-β-D-ribofuranosylimidazo[1,2-*a*]purine) (2). Guanosine (1) (850 mg, 3 mmol) and sodium chloride (3 g) in 750 mL of water were stirred magnetically under nitrogen at 37 °C bath temperature. After most of the guanosine had dissolved (~30 min), 20 mL of 2 M aqueous chloroacetaldehyde solution was added and the pH was carefully maintained between 6.35 and 6.45 using 0.2 M aqueous sodium hydroxide solution in the reservoir of a pH stat. Progress of the reaction was monitored using UV spectra at pH 7. The reaction was stopped when the absorption intensities at λ_{max} 272 and 254 nm became equal. Depending on the concentration of the particular batch of the chloroacetaldehyde and small variations in maintaining the pH and temperature of the reaction, it may take 3–7 days to reach this optimum point. Formation of 1,N^2-ethenoguanosine could be detected by TLC on cellulose in solvents 4 and 3 used successively. At the optimum point a 5-μL spot of the reaction mixture gave a faintly observable nonfluorescent spot

of ethenoguanosine (R_f 0.5, Guo R_f 0.4 in solvent 3). A comparison of NMR signal intensities at δ 7.94 (8-H of Guo) with those at 7.43 or 7.62 (etheno doublets in ε-Guo) in the NMR spectrum of the reaction mixture suggested the presence of about 45% ε-guanosine and 55% unreacted guanosine at the optimum point.

For isolation of 1,N^2-ethenoguanosine, the reaction mixture was evaporated to a syrup at 37 °C under vacuum, dissolved in 200 mL of water, and the aqueous solution was washed three times with equal volumes of ether. The aqueous layer was then concentrated to a small volume, adsorbed over 1 g of cellulose (CF-11), and chromatographed on 500 g of cellulose (column size, 6.4 × 1000 cm) in solvent 1 to yield 50 mg of crude ε-guanosine and 50 mg of a guanosine and ε-guanosine mixture. The latter was rechromatographed on cellulose in solvent 3 to yield an additional 20 mg of ε-guanosine (70 mg isolated, 7.5%, or 17% yield based on the amount of unreacted guanosine). An analytical sample was obtained after three recrystallizations from water (60 °C) as microneedles, mp 252–253 °C (determined on a hot stage microscope). A better yield of very pure product could be obtained by a preliminary purification on a short cellulose column in solvent 1 followed by HPLC on cation exchange resin Aminex A-5 (Bio-Rad) at 50 °C (column 1.3 × 76.2 cm, 0.1 M ammonium formate, pH 4.1, 0.60 mL/min): NMR [$(CD_3)_2SO$] δ 5.85 (d, 1, J = 5 Hz, anomeric H), 7.43 and 7.62 (d, J = 2.5 Hz, etheno H's), 8.16 (s, 1, 2-H); λ_{max} (0.1 M HCl) 222 nm (ε 26 500), 272 (8200), 295 (8300); λ_{max} (pH 7.0) 227 (34 400), 284 (11 900); λ_{max} (0.1 M NaOH) 233 (32 000), 280 (5750), 308 (8840); mass spectrum (70 eV) m/e 175 (base peak), 307 (M^+).

Anal. Calcd for $C_{12}H_{13}N_5O_5$: C, 46.91; H, 4.26; N, 22.79. Found: C, 46.86; H, 4.29; N, 22.86.

1,N^2-Ethenoguanine (5,9-Dihydro-9-oxoimidazo[1,2-*a*]purine) (3). The glyoxal–guanine adduct 5[34] (1.4 g crude, 7 mmol) and hydriodic acid (30 mL, 47%) were stirred magnetically in a stoppered flask at 55–60 °C. The reaction was followed by UV in 0.1 M NaOH until the maximum at 284 nm, due to the glyoxal adduct, was completely replaced by that of the ε-guanine near 315 nm. The reaction was complete in 5–6 days when most of the product precipitated. The mixture was cooled overnight and filtered to yield 820 mg of a cream-colored solid. On concentration, the mother liquor yielded an additional 200 mg (total yield 1.02 g, 41%) of the 1,N^2-ethenoguanine hydriodide, mp > 300 °C. The compound was dissolved in boiling water, and the pH was adjusted to 6–7, causing the crystallization of 0.51 g of the free base·¼H_2O, mp > 290 °C. Anhydrous free base for analysis was obtained by special drying at >200 °C: λ_{max} (0.1 M HCl) 221 nm (ε 29 000), 268 (5750), 295 (8450); λ_{max} (pH 7.0) 224 (39 700), 290 (9700); λ_{max} (0.1 M NaOH) 234 (46 200), 262 (4500), 318 (8900); mass spectrum (70 and 10 eV) m/e 175 (M^+, base peak).

Anal. Calcd for $C_7H_5N_5O$: C, 48.02; H, 2.88; N, 39.98. Found: C, 48.11; H, 3.03; N, 40.00.

The *hydriodide* had the following NMR spectrum [$(CD_3)_2SO$]: δ 7.66 (d, 1, J_{67} = 2.6 Hz, 6- or 7-H), 7.79 (d, 1, J_{67} = 2.6 Hz, 7- or 6-H), 9.37 (s, 1, 2-H), 11.45 (br NH protons, exchanged by D_2O).

Anal. Calcd for $C_7H_6IN_5O$: C, 27.74; H, 2.00; N, 23.11. Found: C, 27.41; H, 1.95; N, 22.45.

Conversion of 1,N^2-Ethenoguanosine (2) to 1,N^2-Ethenoguanine (3). 1,N^2-Ethenoguanosine (10 mg) was treated with 5 mL of 2 M HCl on a steam bath for 1 h. The product was identified as 1,N^2-ethenoguanine by comparison of its UV spectra in acid, base, and neutral media, by co-chromatography with an authentic sample on cellulose TLC plates in two solvent systems (2 and 4), and by its mass spectrum (70 and 10 eV): m/e 175 (M^+, base peak), with no peaks above 175.

***O*⁶-Benzyl-N^2,3-ethenoguanine (9-Benzyloxyimidazo[2,1-*b*]purine) (7a).** O^6-Benzylguanine[37] [NMR[$(CD_3)_2SO$] δ 5.5 (s, 2, CH_2), 6.25 (br s, 2, NH_2, exchanged by D_2O), 7.3–7.6 (m, 5, C_6H_5), 7.83 (s, 1, 8-H); λ_{max} (0.1 M HCl) 233 nm (sh) (ε 5200), 287 (11 000); λ_{max} (pH 7) 239 (7800), 281 (8200); λ_{max} (0.1 M NaOH) 245 (sh) (4600), 284 (8500)] (241 mg, 1 mmol) in 20 mL of 75% aqueous ethanol was treated with 2 mL of 2 M aqueous chloroacetaldehyde solution at pH 4.0–4.5 and 37 °C bath temperature. The reaction was complete in 50–60 h. The mixture was evaporated, the residue was triturated with 5 mL of water, the pH was adjusted to neutral, and the precipitated free base was collected by filtration; 250 mg (94%). Recrystallization from aqueous ethanol provided 220 mg of very fine shiny crystals, mp 243–244 °C: NMR (hydrochloride) [$(CD_3)_2SO$] δ 5.74 (s, 2, CH_2), 7.25–7.7 (m, 5, C_6H_5), 8.08 (d, 1, J_{56} = 2.6 Hz, 6-H), 8.38 (d, 1, J_{56} = 2.6 Hz, 5-H), 8.68 (s, 1, 2-H); NMR (free base) [$(CD_3)_2SO$] δ 5.58 (s, 2, CH_2), 7.30–7.65 (m, 5, C_6H_5), 7.38 (d, 1, J_{56} = 1.5 Hz, 6-H), 7.84 (d, 1, J_{56} = 1.5 Hz, 5-H), 8.20 (s, 1, 2-H); λ_{max} (0.1 M HCl) 216 nm (ε 34 400), 266 (14 950); λ_{max} (pH 7.0) 219 (29 050), 273 (10 550); λ_{max} (0.1 M NaOH) 225 (32 500), 276 (9450); mass spectrum (10 eV) m/e (rel abundance) 265 (M^+), 175 (100); (70 eV) 265 (M^+), 175 (99) ($M^+ - 91 + 1$), and 91 (100) (tropylium ion).

Anal. Calcd for $C_{14}H_{11}N_5O$: C, 63.39; H, 4.18; N, 26.40. Found: C, 63.48; H, 4.14; N, 26.63.

***N*²,3-Etheno-*O*⁶-methylguanine (9-Methoxyimidazo[2,1-*b*]purine) (7b).** O^6-Methylguanine[39] [NMR[$(CD_3)_2SO$] δ 3.97 (s, 3, CH_3), 7.82 (s, 1, 8-H)] (200 mg, 1.2 mmol) suspended in 12 mL of aqueous ethanol (60%) was treated with 1.5 mL of 2 M aqueous chloroacetaldehyde solution at pH 4.0–4.5 and 37 °C bath temperature and the product was isolated as in the reaction of O^6-benzyl-N^2,3-ethenoguanine. Recrystallization from a large excess of boiling water afforded 185 mg (81%) of fine white crystals (¼ H_2O), mp 228–230 °C. Special drying above 110 °C provided the anhydrous free base for analysis: NMR (hydrochloride) [$(CD_3)_2SO$] δ 4.24 (s, 3, CH_3), 8.06 (d, 1, J_{56} = 2.6 Hz, 6-H), 8.38 (d, 1, J_{56} = 2.6 Hz, 5-H), 8.67 (s, 1, 2-H); NMR (free base) [$(CD_3)_2SO$] δ 4.1 (s, 3, CH_3), 7.44 (d, 1, J_{56} = 1.6 Hz, 6-H), 7.86 (d, 1, J_{56} = 1.6 Hz, 5-H), 8.29 (s, 1, 2-H); λ_{max} (0.1 M HCl) 215 nm (ε 26 850), 264–269 (br) (12 200); λ_{max} (pH 7.0) 221 (24 500), 272 (9900); λ_{max} (0.1 M NaOH) 225 (29 550), 275 (8800); mass spectrum (70 and 10 eV) m/e 189 (M^+ and base peak).

Anal. Calcd for $C_8H_7N_5O$: C, 50.79; H, 3.73; N, 37.02. Found: C, 50.69; H, 3.81; N, 37.47.

***N*²,3-Ethenoguanine (8,9-Dihydro-9-oxoimidazo[2,1-*b*]purine) (8).** O^6-Benzyl-N^2,3-ethenoguanine (7a) (100 mg) was treated with 10 mL of 2 M hydrochloric acid on a steam bath for 1 h. The solution was evaporated to dryness and the residue was triturated with ether and filtered. The solid was dissolved in boiling water, the pH was adjusted to 6–7, and the free base (¼ H_2O) crystallized: yield, 58 mg (88%) of white crystals; mp > 290 °C; NMR (hydrochloride) [$(CD_3)_2SO$] δ 7.61 (d, 1, J_{56} = 2.2 Hz, 6-H), 8.02 (d, 1, J_{56} = 2.2 Hz, 5-H), 8.38 (s, 1, 2-H); λ_{max} (0.1 M HCl) 215 nm (ε 25 000), 256 (9650); λ_{max} (pH 7.0) 216 (28 200), 262 (12 850); λ_{max} (0.1 M NaOH) 229 (32 000), 270 (9600); mass spectrum (10 and 70 eV) 175 (M^+, base peak). Anhydrous free base was obtained for analysis by special drying >200 °C.

Anal. Calcd for $C_7H_5N_5O$: C, 48.02; H, 2.88; N, 39.98. Found: C, 47.93; H, 2.80; N, 39.92.

Debenzylation of O^6-Benzyl-N^2,3-ethenoguanine by Catalytic Hydrogenolysis. O^6-Benzyl-N^2,3-ethenoguanine (7a) hydrochloride (100 mg) was hydrogenated at 25 °C and 1 atm in the presence of 10% Pd/C in 10 mL of absolute ethanol for 11 h. The mixture was diluted with 100 mL of 75% aqueous ethanol and filtered. The filtrate was evaporated to dryness, and the residue was recrystallized from a large excess of boiling water at neutral pH to yield 45 mg (68%) of N^2,3-ethenoguanine (8) identical in all respects with that obtained by acid hydrolysis of 7a.

Demethylation of 7b. N^2,3-Etheno-O^6-methylgaunine (7b) was converted quantitatively to N^2,3-ethenoguanine on heating with concentrated HCl on a steam bath for 24 h. The product was identified by comparison of its UV, NMR, and mass spectra (see above).

6-Benzylthio-N^2,3-ethenoguanine (9-Benzylthioimidazo-[2,1-*b*]purine) (9). 2-Amino-6-benzylthiopurine[40,41] [NMR [$(CD_3)_2SO$] δ 4.54 (s, 2, CH_2), 6.38 (br s, 2, Pu-NH_2, exchanged by D_2O), 7.2–7.6 (m, 5, C_6H_5), 7.88 (s, 1, 8-H)] (600 mg, 2.34 mmol) was treated with 3.5 mL of 2 M aqueous chloroacetaldehyde solution in 60 mL of 75% aqueous ethanol at pH 4–4.5 and 37 °C bath temperature. The reaction was complete in 6–7 days. The pH was adjusted to neutral using 1 M $NaHCO_3$ solution, and the precipitated product was filtered, washed with water twice, and dried to yield an off-white powder (626 mg, 93%). It was recrystallized from a large excess of boiling water to give shiny cream-colored, fine crystals: mp 295 °C; NMR [$(CD_3)_2SO$ + DCl] δ 4.74 (s, 2, CH_2), 7.2–7.7 (m, 5, C_6H_5), 8.14 (d, 1, J_{56} = 2.4 Hz, 6-H), 8.38 (d, 1, J_{56} = 2.4 Hz, 5-H), 8.7 (s, 1, 2-H); NMR (free base) [$(CD_3)_2SO$] δ 4.67 (s, 2, CH_2), 7.2–7.55 (m, 5, C_6H_5), 7.61 (d, 1, J_{56} = 1.5 Hz, 6-H), 7.97 (d, 1, J_{56} = 1.5 Hz, 5-H), 8.26 (s, 1, 2-H); λ_{max} (0.1 M HCl) 301 nm (24 700); λ_{max} (pH 7.0) 302 (14 100); λ_{max} (0.1 M NaOH) 248 (14 800), 300 (18 800); mass spectrum (10 eV) m/e (rel abundance) 281 (M^+ and base peak); (70 eV) 281 (60) (M^+), 248 (60) ($M - SH)^+$, 91 (100).

Anal. Calcd for $C_{14}H_{11}N_5S$: C, 59.77; H, 3.94; N, 24.89. Found: C, 59.68; H, 3.90; N, 24.99.

Conversion of 6-Benzylthio-N^2,3-ethenoguanine to N^2,3-Ethenoguanine. 6-Benzylthio-N^2,3-ethenoguanine (9) (17 mg) was treated with 24 mg of *N*-chlorosuccinimide in 5 mL of aqueous methanol (80%) at 50–60 °C. In <1 h a highly fluorescent solution resulted. The reaction was allowed to proceed overnight, the solution was evaporated to dryness, and the residue was purified by chromatography over a short silica gel column in solvent 6. The fractions containing the oxidized compound (UV detection) were pooled and evaporated. The residue was treated with 2 N HCl overnight on a steam bath. The product was purified by HPLC on cation exchange

resin Aminex A-5 (Bio-Rad) at 50 °C (column 1.3 × 76.2 cm, 0.1 M ammonium formate, pH 4.1, 0.60 mL/min) and was identified as N^2,3-ethenoguanine by its HPLC elution volume, UV, and mass spectrum.

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