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ELECTROPHILIC AMINATION OF GUANINE DERIVATIVES. MECHANISM FOR FORMATION OF 8-AMINOGUANINE DERIVATIVES

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Abstract A study of the mechanism of 8-aminoguanosine formation in the reaction of guanosine with hydroxylamine-O-sulfonic acid (HAOS) revealed that the reaction proceeds in three steps, 1) electrophilic N-7 amination by HAOS, 2) nucleophilic C-8 hydroxyamination by NH_2OH accompanied by elimination of the N-7 amino group and by aromatization, and finally 3) the reduction of the C-8 hydroxyamino group by NH_2OH to give 8-aminoguanosine The significance of formation of the 7-aminoguanosine intermediate is discussed briefly in relation to the reaction of carcinogenic arylamines and arylhydroxylamines with cellular DNA

Carcinogenic arylamines and arylhydroxylamines are known to be metabolically activated to N-aryl-O-acylhydroxylamines which are capable of modifying cellular DNA resulting in chemical carcinogenesis However, reactivity of the ultimate forms of the carcinogens toward nucleic acid components is poorly understood To clarify the mechanisms involved, we have been studying the reaction of nucleosides with the simple electrophilic aminating agents, hydroxylamine-O-sulfonic acid (HAOS) and 2,4-dinitrophenoxyamine (DNPA) [1-7] These are expected to react via the same type of reaction pathway as those involved in aminations by carcinogenic arylamines and arylhydroxylamines Our previous study showed that the reaction of guanosine with HAOS at pH 2 to 4 yielded 8-aminoguanosine,[1] the aminated position of which is the same as that of the major guanine-carcinogen adducts generally formed in cellular DNA treated with the carcinogens In this report, we describe the mechanism of 8-amino-guanosine formation and briefly describe a mechanism of DNA modification by the carcinogens concerned

Reaction of guanosine (**1a**) with 10 eq mol of HAOS at 70°C and pH 2 to 4 for 2 h gave 8-aminoguanosine (**2a**) in 20% yield in addition to the recovered guanosine, as reported previously [1] (Scheme I) Although the C-8 position of guanosine is reported to be reactive toward either electrophiles or radicals,[8] details of the mechanism are not clear in most cases When deoxyguanosine was subjected to amination, 7-aminoguanine and 8-aminoguanine were obtained in 20% and 5% yields, respectively, together with recovered deoxyguanosine and its deglycosilated guanine This result, together with our previous finding that 7-aminoguanosine is very reactive toward nucleophiles,[5,6] suggested that amination at C-8 of the guanine nucleus might proceed via the initial amination at the N-7 position Then, 7-aminoguanosine (**3a**), which was prepared from guanosine and DNPA in N,N-dimethylformamide (DMF) using our reported method,[5] was tested for its subsequent conversion to 8-aminoguanosine (**2a**) (Scheme

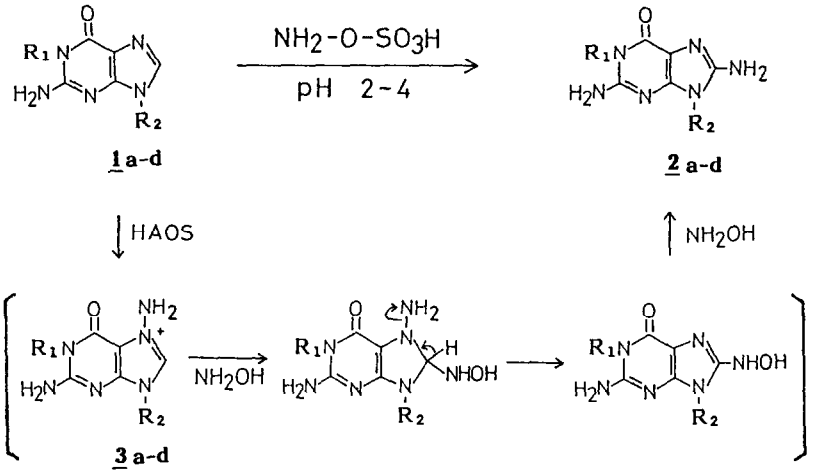
II). As expected, 8-aminoguanosine was formed when 7-aminoguanosine was treated with HAOS under the same reaction conditions as employed for the amination of guanosine It was further shown that $NH_2OH \cdot HCl$ could be substituted for HAOS in the conversion of 7-aminoguanosine to 8-amino derivatives This is unequivocal evidence to support the fact that hydroxylamine, liberated by hydrolysis of HAOS during the reaction with guanosine, took part in converting 7-aminoguanosine to 8-aminoguanosine Since hydroxylamine is a nucleophile and also a powerful reducing agent, one can demonstrate the reaction mechanism involved as shown in Scheme I HAOS aminates the most nucleophilic N-7 position of guanosine (**1a**) in an electrophilic manner to yield 7-aminoguanosine (**3a**), which undergoes nucleophilic attack by hydroxylamine (or $NH_2OSO_3^-$) to form the 7,8-dihydro-7-amino-8-hydroxyamino intermediate, followed by aromatization to 8-hydroxyaminoguanosine, accompanied by elimination of the 7-amino group Then an excess of hydroxylamine, the hydrolyzed product of HAOS, reduces 8-hydroxyamino-guanosine to 8-aminoguanosine (**2a**) as the isolated product The ready reduction of 8-hydroxy-aminoguanosine to 8-aminoguanosine is strongly supported by the evidence obtained from the reaction of 8-bromoguanosine with NH_2OH, the reaction yielded mainly 8-aminoguanosine and guanosine in a ratio of 5 3 1, with only a trace amount of 8-hydroxyaminoguanosine [9] A similar amination reaction using NH_2OH is described elsewhere [10]

The mechanism stated above was further confirmed by ^{15}N-analysis of the product obtained by treatment of 7-aminoguanosine with $^{15}NH_2OH \cdot HCl$ The isolated product was 8-$^{15}NH_2$-guanosine, the structure of which was confirmed by comparison of the spectral data with those of an authentic specimen When $CH_3NHOH \cdot HCl$ was allowed to react with 7-aminoguanosine, the product obtained was 8-methylaminoguanosine This result also supports the proposed mechanism

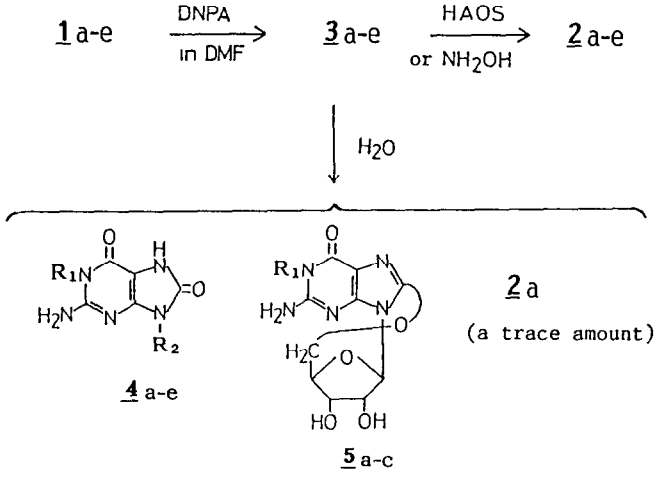
7-Aminoguanine formation in the reaction of deoxyguanosine with HAOS at pH 2 to 4 is readily understood, the cationic 7-aminodeoxyguanosine formed is easily deglycosylated to stable 7-aminoguanine under acidic conditions before it is attacked at the C-8 position by hydroxylamine

The narrow optimal pH range (2 to 4) for 8-aminoguanosine formation in the reaction of guanosine with HAOS is discussed For the formation of 7-aminoguanosine in the initial step, the free form of guanosine (p*K*a at N-7, 1 9) is required, therefore, the optimal pH is not much below 1 9 For the conversion of 7-aminoguanosine to 8-aminoguanosine in the second step, a higher pH is unfavorable, because as we previously reported, 7-aminoguanosine readily undergoes, even in neutral media, nucleophilic attack by OH^- and intramolecular 5'-O^-, accompanied by elimination of the 7-amino group, to yield 8-hydroxyguanosine [11] and 8,5'-anhydro-8-hydroxyguanosine (8,5'-*O*-cycloguanosine), respectively [5] In contrast, the nucleophilic attack of NH_2OH (p*K*a, 6 03) on 7-aminoguanosine involved in the second step proceeds slower as the pH decreases under 6 03 In this step, nucleophilic $NH_2OSO_3^-$ may also take part in forming the 8-NHOH adduct *via* the 8-$NHOSO_3H$ adduct since HAOS has the ability to act both as electrophile and nucleophile As a compromise of the above requirements, the optimal pH is allowed to range from 2 to 4 In fact, when the reaction of guanosine with HAOS was carried out at pH 6 to 8, 1-aminoguanosine, 8-hydroxyguanosine

Scheme I



Scheme II



a R_1= H, R_2= β-D-ribofuranosyl
b R_1= CH_3, R_2= β-D-ribofuranosyl
c R_1= NH_2, R_2= β-D-ribofuranosyl
d R_1= NH_2, R_2= CH_3
e R_1= H, R_2= CH_3

and 8,5'-anhydro-8-hydroxyguanosine were produced without the formation of 8-aminoguanosine In media at pH 6 to 8, attacks by OH^- and intramolecular 5'-O^- at the C-8 position of 7-aminoguanosine might have predominated over that of hydroxylamine.

Some guanine derivatives other than guanosine and deoxyguanosine were subjected to the electrophilic amination reaction described above (Scheme I) Treatment of 1-amino-guanosine (**1c**) with HAOS at pH 2 to 4 yielded the 8-aminated derivative (**2c**) as well 1,7-Diaminoguanosine is thought to be the reaction intermediate for converting to the 8-amino derivative When 1-amino-9-methylguanine (**1d**) was treated with HAOS at pH 2 to 4, the main product was 1,7-diamino-9-methylguanine (**3d**) and not the expected product, 1,8-diamino-9-methylguanine The structure of **3d** was confirmed by comparison with an authentic sample prepared from the reaction of **1d** with DNPA in DMF Compound **3d** was more stable toward nucleophilic attack than the corresponding 9-ribosyl derivatives such as 7-aminoguanosine and 1,7-diaminoguanosine In fact, more vigorous conditions were required for conversion of 1,7-diamino-9-methylguanine (**3d**) to 1,8-diamino-9-methylguanine (**2e**) and 1-amino-8-hydroxy-9-methylguanine (**4c**) by reacting with NH_2OH and H_2O, respectively It is worth noting that the susceptibility of the C-8 position of 7-aminoguanine derivatives to nucleophiles is dependent on the 9-substituent, a ribosyl or a methyl

For a model of 7-aminoguanosine, 1-methyl-3-aminobenzimidazole was synthesized and the reactivity of the C-2 position toward nucleophiles was studied 1-Methyl-3-amino-benzimidazole was so stable that it yielded neither 1-methyl-2-hydroxybenzimidazole nor 1-methyl-2-aminobenzimidazole after reacting with H_2O or NH_2OH This may be due to the higher electron density of the imidazole ring of 1-methyl-3-aminobenzimidazole compared to that of 7-aminoguanosine

Syntheses of several 7-aminoguanine derivatives and their conversion to 8,5'-O-cyclo and/or 8-hydroxy derivatives by treatment with hot H_2O are summerized in Scheme II It is worth noting that in conversion of 7-aminoguanosine to 8-hydroxyguanosine, a trace amount of 8-aminoguanosine was formed Another mechanism could work in practice unless an excess of hydroxylamine is present in the reaction media As a probable mechanism, the attack of OH^- at the C-8 position of 7-aminoguanosine forms 7,8-dihydro-7-amino-8-hydroxyguanosine intermediates, followed by the elimination of the OH group and the concerted cyclization to a diaziridine intermediate, which leads to formation of the 8-amino derivative This mechanism is reminiscent of our recent finding that the 8-arylaminated guanine adduct is formed together with the 8-hydroxyguanine residue in cellular DNA treated with carcinogenic 4-hydroxyamino-quinoline 1-oxide [12,13] This suggests that such a mechanism possibly operates in DNA modifications by carcinogenic arylamines and arylhydroxylamines in general Further study in this area is in progress

EXPERIMENTAL

^1H NMR spectra were recorded on a JEOL FX-100 spectrometer Chemical shifts are expressed in parts per million relative to Me_4Si Mass spectra were obtained on a JEOL

DX-300 spectrometer, and samples of nucleosides were trimethylsilylated with N,O-bis(trimethylsilyl)trifluoroacetamide - 1% trimethylsilyl chloride in pyridine before use UV spectra were obtained on a Shimadzu UV-2100 spectrophotometer TLC (cellulose) and paper chromatography (Whatman 3 MM) were carried out using the solvent system (isopropanol 1% aqueous NH_4SO_4 = 3 2, v/v) unless otherwise specified

Reaction of deoxyguanosine with HAOS Deoxyguanosine (600 mg, 2 25 mmol) was dissolved in 25 mL of H_2O containing 2 87 g of CH_3COOK, and the solution was kept at 70°C Then, HAOS (2 54 g, 22 5 mmol) in 25 mL H_2O was added in small portions to the solution During the reaction, the pH of the reaction mixture was maintained at 2 to 4 by further addition of concentrated CH_3COOK solution After 2 h, products were separated by Whatman 3MM paper chromatography and/or cellulose TLC and further purified on an LH 20 column eluted with H_2O Products obtained were 7-aminoguanine[7] (about 20% yield, Rf on cellulose TLC, 0 32), 8-aminoguanine (about 5% yield, Rf 0 21), guanine (Rf 0 43), and recovered deoxyguanosine (Rf 0 66) Structures of the products were confirmed by spectral data compared with those of authentic samples

Reaction of 7-aminoguanosine with HAOS, NH_2OH, $^{15}NH_2OH$, or CH_3NHOH Cationic 7-aminoguanosine was prepared by the reaction of guanosine with DNPA as described previously[5] The 7-aminoguanosine obtained contained small amounts of 8-hydroxyguanosine and 8,5'-anhydro-8-hydroxyguanosine, which were formed during the separation of 7-amino-guanosine First, the following two preliminary experiments were carried out A) 7-Amino-guanosine (5 mg) and 10 eq mol of HAOS in 1 mL of H_2O (pH 2 to 4) were heated at 70°C for 2 h B) 7-Aminoguanosine (5 mg) and 10 eq mol of pre-heated HAOS solution (1 mL), which was prepared by heating HAOS solution at 70°C and pH 2 to 4 for 2 h, were mixed and the solution was heated at 70°C for 2 h It was shown that pre-heated HAOS had no aminating ability when it was allowed to react with guanosine After experiments A and B, products were analyzed by cellulose TLC The results showed that 8-aminoguanosine (Rf 0 34) was the main product in both experiments Next, 7-aminoguanosine (10 mg) was dissolved in 2 mL of H_2O and the pH of the solution was kept at 2 to 4 by addition of conc CH_3COOK solution Then, 10 eq mol of $NH_2OH \cdot HCl$, $^{15}NH_2OH$ HCl, or CH_3NHOH HCl was added, and the mixture was heated at 70°C for 2 h maintaining the pH of the reaction mixture at 2 to 4 Products were separated by cellulose TLC and purified further on an LH 20 column eluted with H_2O The yield of 8-amino- or 8-methylaminoguanosine was around 60 to 70%, and others were 8-hydroxy and 8,5'-O-cyclo derivatives The structures of 8-amino-, 8-^{15}N-amino-, and 8-methylaminoguanosines were identified by NMR, mass, and UV spectra compared with those of the corresponding authentic samples Rf values of the products on cellulose TLC were 7-aminoguanosine[5] (0 25), 8-aminoguanosine[14,16] (0 34), 8,5'-anhydro-8-hydroxyguanosine[5] (0 37), 8-hydroxyguanosine[14,16] (0 52), and 8-methylamino-guanosine[15,16] (0 59)

Authentic 8-^{15}N-aminoguanosine One gram of $^{15}NH_2$-$^{15}NH_2 \cdot H_2SO_4$ (MSD Isotopes, Montreal, Canada) was added to a solution of sodium hydroxide (0.63 g) in 3 mL of H_2O. From the solution, the mixture of $^{15}NH_2$-$^{15}NH_2 \cdot H_2O$ and H_2O (total 2 8 mL) was separated by use of a micro distillation apparatus (180°C, 30 mmHg) After 8-bromoguanosine (80 mg) and 0 5 mL of H_2O were added to this $^{15}NH_2$-$^{15}NH_2$ solution, the mixture was heated at 95°C for 24 h TLC of the reaction mixture on a cellulose plate showed two products No starting material (Rf 0 63) was observed 8-$^{15}NH_2$-Guanosine (Rf 0 34) and guanosine (Rf 0 52) were obtatned at a product ratio of 2 to 3 8-$^{15}NH_2$-Guanosine was isolated by repeated TLC separations, and further purified on an LH 20 column eluted with H_2O ^{1}H NMR (JEOL GX 400 MHz, DMSO-d_6) δ 3 61 (m, 2H, 5'-H), 3 87 (q, 1H, 4'-H), 4 08 (m, 1H, 3'-H), 4 54 (q, 1H, 2'-H), 5.01 (br d, 1H, 3'-OH), 5 24 (br d, 1H, 2'-OH), 5 50 (br t, 1H, 5'-OH), 5 73 (d, 1H, J = 7 4 Hz, 1'-H), 6 06 (br d, 2H, J = 84 4 Hz, 8-$^{15}NH_2$), 6 22 (br s, 2H, 2-NH_2), 10 62 (br s, 1H, NH) The UV spectra in acidic, neutral and alkaline media were identical to that of 8-aminoguanosine

Reaction of guanosine with HAOS at pH 6 to 8 Guanosine (600 mg, 2 12 mmol) was dissolved in 25 mL of H_2O containing 2 70 g of CH_3COOK, and the solution was kept at 70°C Then, HAOS (2 40 g, 21.2 mmol) in 25 mL H_2O was added in small portions to the solution During the reaction, the pH was kept between 6 to 8 by addition of con NaOH solution After 2 h, product analysis was carried out by comparing Rf values with those of authentic samples on a cellulose TLC plate. Products obtained were 8-hydroxyguanosine (Rf 0 52), 1-amino-guanosine (Rf 0 43), and 8,5'-anhydro-8-hydroxyguanosine (Rf 0 37)

1-Amino-9-methylguanine 9-Methylguanine (1 32 g, 8 00 mmol) was dissolved in 20 mL of H_2O containing NaOH (1 28 g, 32 0 mmol) HAOS (1 80 g, 15 9 mmol) was added in small portions with stirring at room temperature After 16 h, the precipitate which appeared was collected by filtration and washed with EtOH and then Et_2O Recrystallyzation of the precipitate from H_2O yielded needles (1 10 g), mp 305-307°C ^{1}H NMR (DMSO-d_6) δ 3 55 (s, 3H, CH_3), 5 40 (br s, 2H, 1-NH_2), 7 14 (br s, 2H, 2-NH_2), 7 76 (s, 1H, 8-H) By addition of D_2O, the 1-NH_2 protons were exchanged with D faster than those of the 2-NH_2 UV λ_{max}nm pH 1, 253 and 279, H_2O and pH 12, 254 and 270(sh) MS m/z = 180 Anal Calcd for $C_6H_8N_6O \cdot 1/5H_2O$ (dried in vacuo at 50°C for 2 h) C, 39 22, H, 4 58, N, 45 75 Found C, 39 50, H, 4 44, N, 45 67

Reaction of 9-methylguanine with DNPA 9-Methylguanine (50 mg, 0 30 mmol) was suspended in 30 mL of DMF, and DNPA (302 mg, 1 52 mmol) was added to the solution The mixture was left at 40°C for 4 days with stirring Because of the low solubility of 9-methylguanine in DMF, the precipitate which separated was almost all the starting material used for the reaction TLC of the mother liquor showed formation of 7-amino-9-methylguanine UV λ_{max}nm pH 1 and H_2O, 253 and 282, pH 12, 268 Rf (cellulose TLC) 9-methylguanine, 0 74, 7-amino-9-methylguanine, 0 50 When DMSO was substituted for DMF, 9-methylguanine

completely dissolved, however, the amination reaction did not proceed at all

Reaction of 1-amino-9-methylguanine with HAOS 1-Amino-9-methylguanine hydrate (184 mg, 1 00 mmol) was dissolved in 12 mL of H_2O containing 1 27 g of CH_3COOK, and the solution was warmed to 70°C HAOS (1 13 g, 10.0 mmol) in 12 mL of H_2O was added dropwise to the solution and the mixture was left at 70°C for 2 h, maintaining the pH between 2 to 4 by addition of conc CH_3COOK sol After the reaction, TLC (cellulose) of the reaction mixture showed the formation of 1,7-diamino-9-methylguanine (20% yield, Rf 0 21) and unreacted 1-amino-9-methylguanine (Rf 0 36) Further heating (100°C, 2 h) of the reaction mixture gave 1,8-diamino-9-methylguanine

Reaction of 1-amino-9-methylguanine with DNPA (Synthesis of 1,7-diamino-9-methylguaninium chloride (**3d**)) To the solution of 1-amino-9-methylguanine hydrate (184 mg, 1 00 mmol) in 15 mL of DMF, DNPA (796 mg, 4 00 mmol) was added, and the mixture was left standing at 45°C for 4 days After the reaction, TLC (cellulose) of the reaction mixture showed quantitative formation of 1,7-diamino-9-methylguaninium chloride (Rf 1-amino-9-methylguanine, 0 36, 1,7-diamino-9-methylguaninium chloride, 0.21) After removal of DMF by evaporation, the residue was dissolved in 20 mL of 1 N HCl and washed with ether (20 mL x 2) The aqueous solution was concentrated to 5 mL by evaporation EtOH-Et_2O was then added to cause precipitation of a product with mp 211-212°C dec ^1H NMR (DMSO-d_6) δ 3 64 (s, 3H, CH_3), 5 57 (s, 2H, 1-NH_2), 6 95 (s, 2H, 2-NH_2), 9 26 (s, 1H, 8-H) 7-NH_2 protons were not observed as a peak The 8-H proton was completely exchanged with D when the compound was heated in D_2O at 60°C for 30 min UV λ_{max}nm pH 1 and H_2O, 253 and 283, pH 12, 266 Anal Calcd for $C_6H_{10}ClN_7O$ C, 31 10, H, 4 35, N, 42 33 Found C, 31 26, H, 4 54, N, 41 82

Reaction of 1,7-diamino-9-methylguanine with H_2O (Synthesis of 1-amino-8-hydroxy-9-methylguanine (**4d**)) 1,7-Diamino-9-methylguaninium chloride (35 0 mg) was dissolved in 3 5 mL of H_2O and the pH of the solution was adjusted to 7 5 with dil NaOH sol The mixture was heated at 70°C for 5 h After cooling, the precipitate which appeared was collected and recrystallized from H_2O to yield a powder (20 mg), mp > 300°C ^1H NMR (DMSO-d_6) δ 3 08 (s, 3H, CH_3), 5 38 (br s, 2H, 1-NH_2), 7 14 (br s, 2H, 2-NH_2), 10 6 (hump, 1H, 7-NH) UV λ_{max}nm pH 1 and H_2O, 248 and 290, pH 12, 260 and 292 MS m/z = 196 Anal Calcd for $C_6H_8N_6O_2$ H_2O (dried in vacuo at 50°C for 2 h) C, 33 64, H, 4 67, N, 39 25 Found C, 33 87, H,4 65, N, 39 34 Rf (cellulose TLC) 0 36

Reaction of 1-aminoguanosine with HAOS (Synthesis of 1,8-diaminoguanosine (**2c**)) 1-Aminoguanosine[17] (600 mg, 2 01 mmol) was treated with HAOS (2 28 g, 20 1 mmol) at pH 2 to 4 as described for the reaction of deoxyguanosine with HAOS After the reaction, TLC (cellulose) of the reaction mixture showed the formation of 1,8-diaminoguanosine (25% yield, Rf 0 26) and recovered 1-aminoguanosine (Rf 0 43) 1,8-Diaminoguanosine was separated by

TLC and paper chromatography and purified on an LH 20 column (H_2O). ^1H NMR (DMSO-d_6) δ 3 60 (m, 2H, 5'-H), 3.86 (m, 1H, 4'-H), 4 09 (m, 1H, 3'-H), 4 54 (m, 1H, 2'-H), 5 01 (br d, 1H, 3'-OH), 5 22 (br d, 1H, 2'-OH), 5 29 (br s, 2H, 1-NH_2), 5 49 (br t, 1H, 5'-OH), 5 73 (d, 1H, J = 7 4 Hz, 1'-H), 5 99 (br s, 2H, 8-NH_2), 6 70 (br s, 2H, 2-NH_2) UV λ_{max}nm pH 1, 251 and 286, H_2O and pH 12, 257 and 288

Reaction of 1-amino- or 1-methylguanosine with DNPA, and conversion of the product to 8-hydroxy and 8,5'-O-cyclo derivatives 1-Aminoguanosine (298 mg, 1 00 mmol) in 20 mL of DMF-H_2O (3 1, v/v) was treated with DNPA (796 mg, 4 00 mmol) as described for the reaction of 1-amino-9-methylguanine with DNPA. The yield of 1,7-diaminoguanosine was about 45% It was isolated by paper chromatography and used without further purification in the subsequent experiment Rf (cellulose TLC) 1-aminoguanosine, 0 43, 1,7-diaminoguanosine, 0 20. 1,7-Diaminoguanosine was heated in H_2O, and products were separated and purified as described above Products were mainly 1-amino-8-hydroxyguanosine and 1-amino-8,5'-anhydro-8-hydroxyguanosine and a trase amount of 1,8-diaminoguanosine Rf (cellulose TLC) 1-amino-8-hydoxyguanosine, 0 43, 1-amino-8,5'-anhydro-8-hydoxyguanosine, 0 29, 1,8-diaminoguanosine, 0 26 1-Amino-8,5'-anhydro-8-hydroxyguanosine ^1H NMR (DMSO-d_6) δ 3.5 - 4 5 (m, 5H, 2'-, 3'-, 4'-, and 5'-H), 4 85 (br d, 1H, 3'-OH), 5 34 (br s, 2H, 1-NH_2), 5 38 (br d, 1H, 2'-OH), 5 73 (s, 1H, 1'-H), 7 10 (br s, 2H, 2-NH_2) UV λ_{max}nm pH 1, 254 and 276(sh), H_2O and pH 12, 252 and 275(sh) MS m/z = 656 (5TMS) Identification of 1-amino-8-hydroxyguanosine[17] and 1,8-diaminoguanosine was performed by comparison of NMR and UV spectra with those of authentic samples In the case of 1-methylguanosine (297 mg, 1 00 mmol), it was dissolved in 15 mL of DMF and then DNPA (796 mg, 4 00 mmol) was added Methods for the reaction and separation were the same as for 1-aminoguanosine TLC (cellulose) of the reaction mixture showed 1-methyl-7-aminoguanosine (90% yield, Rf 0 17) and recovered 1-methylguanosine (Rf 0 60) 1-Methyl-7-aminoguanosine was separated and heated in H_2O as described above Products were 1-methyl-8-hydroxyguanosine (Rf 0 60) and 1-methyl-8,5'-anhydro-8-hydroxyguanosine (Rf 0 30). 1-Methyl-8,5'-anhydro-8-hydroxyguanosine ^1H NMR (DMSO-d_6) δ 3 28 (s, 3H, CH_3), 3 4-4 6 (m, 5H, 2'-, 3'-, 4'-, and 5'-H), 5.2 - 5 6 (hump, 2H, 2'- and 3'-OH), 5 73 (s, 1H, 1'-H), 7 04 (br s, 2H, NH_2) UV λ_{max}nm pH 1, 254 and 275(s), H_2O and pH 12, 253 and 275(sh). MS m/z = 511 (3TMS) 1-Methyl-8-hydroxyguanosine UV λ_{max}nm pH 1 and H_2O, 249 and 290, pH 12, 261 and 297 MS m/z = 601 (4TMS)

Reaction of 1-methylbenzimidazole with HAOS or DNPA 1-Methylbenzimidazole (20 mg, 0 15 mmol) and DNPA (119 mg, 0 60 mmol) were dissolved in 2 mL of DMF, and the reaction mixture was left standing at 40°C for 12 h TLC (silica gel, $CHCl_3$ MeOH = 9 1, v/v) showed a quantitative formation of 1-methyl-3-aminobenzimidazole (Rf 0 05) Rf of 1-methylbenzimidazole was 0 28 After the solvent was removed by evaporation, 2 mL of dil HCl was added to the residue, and the solution was washed with Et_2O (5 mL x 3) and AcOEt (5 mL x 3) The aqueous layer was then evaporated to dryness and the product was purified on an LH 20 column eluted with MeOH Next, 1-methylbenzimidazole (20 mg, 0 15 mmol)

and CH_3COOK (191 mg) were dissolved in 2 mL of H_2O, and the mixture was heated at 70°C Then, HAOS (170 mg, 1 5 mol) in 2 mL of H_2O was added dropwise to the solution and the reaction mixture was left at 70°C for 3 h, maintaining the pH of the solution between 6 and 7 TLC (silica gel, $CHCl_3$ MeOH = 9 1, v/v) of the reaction mixture showed the formation of 1-methyl-3-aminobenzimidazole (20% yield, Rf 0 05) and unreacted starting material (Rf 0 28) The structure of 1-methyl-3-aminobenzimidazole[18] was identified by comparison with an authentic sample When the reaction was carried out between pH 2 to 4, no amination reaction proceeded due to protonation (pKa, 5 6).

Reaction of 1-methyl-3-aminobenzimidazole with H_2O 1-Methyl-3-aminobenzimidazolium chloride (10 mg) was dissolved in 20 mL of H_2O and the pH of the solution was adjusted to 7 0 with dil. NaOH sol The mixture was heated at 70°C for 3 h Under these conditions, 1-methyl-3-aminobenzimidazole was stable and did not yield any products However, when the reaction was carried out at pH 12, many products were identified by TLC Further product analyses were not carried out

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