Synthesis of some modified guanosine derivatives

M S Amer*, A M Amer, A F Sayed Ahmed & W M Farouk
Chemistry Department, Faculty of Science, Zagazig University, Zagazig-Egypt

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Synthesis of some modified guanosine derivatives having substitution at 8-position by some amino compounds such as diphenylamine, ethylhydrazinoformate, serine, proline and glycine have been described. Furthermore, 2,3-O-isopropylidene-8-bromoguanosine reacts with dimethylamine to give 2,3-O-isopropylidene-8-(N, N-dimethyl)aminoguanosine. The later compound is used for the synthesis of 2,5-anhydronucleoside. The cyclonucleoside is treated with H_2S in dry pyridine to give 5-thioguanosine derivative 11. The biological activity of the synthesized compounds have also been measured.

Modified guanosines are present in some positions of tRNAs^{14}. These nucleosides play an important role in transfer of the genetic code. Many of these modifications result from methylation in the base at O-2 of ribose but there are also a number of hypermodified guanosines which reflect more complex modifications. The methylated guanosine (MG) is present in two positions 9 and 37 in eukaryotic tRNAs. More than 280 known tRNA Sequences^{38} reveal that the anticodon loop is especially rich in modification. It includes hypermodified derivatives of uridine and guanosine in the first wobble position of the anticodon and hypermodified derivatives of adenosine and guanosine in the position adjacent to its 3-end. It was reported that a series of 8-substituted guanosine and 2-deoxyguanosines derivatives were tested as inducers for the differentiation of Friend murine erythro-leukemia cells in culture^{9}. It is notable that derivatives containing amino acid residues play an important role in codon and anticodon interaction during protein biosynthesis^{10-12}. The present study involves the synthesis of some modified guanosine derivatives which include substitution at the 8-position of the heterobase ring. The biological activity of the synthesized compounds were tested using the cup-plate technique. Bromoguanosine 7 and 2,3-O-isopropylidene-8-bromoguanosine 8^{13,14} were used as suitable substrates for the synthesis of the desired guanosine derivatives. The coupling reactions of the substrate 7 with some appropriate compounds such as diphenylamine, ethylhydrazinoformate, serine, proline and glycine have been accomplished in dimethylformamide and in moderate conditions to afford the corresponding products in reasonable yields. Each reaction was monitored by thin layer chromatography and the products were crystallized from a mixture of methanol-chloroform. Furthermore, 2,3-O-isopropylidene-8-bromoguanosine 8 was reacted with dimethylamine to give 2,3-O-isopropylidene-8-(N, N-dimethyl)aminoguanosine 9. The latter compound was chromatographed on silica gel column. It was important to select a suitable procedure for the synthesis of 2,5-anhydronucleoside 10. In principle, the formation of N_25-cyclonucleoside should be possible whenever a reactive leaving group is generated at C-5 of ribose moiety in the absence of an effective external nucleophile. A suitable method developed by Wada and Mitsunobu^{15} consists of the reaction of 2,3-O-isopropylidene-8-(N, N-dimethylamino) guanosine with triphenylphosphine and diethylazadicarboxylate. In the first step of the reaction a quaternary phosphonium salt is produced. The second step consists of the abstraction of the hydrogen atom from the amino group at C-2 of guanosine derivative by diethylazadicarboxylate followed by intramolecular electron transfer to give the desired cyclonucleoside. The advantage of this (one-pot) procedure lies in the high yield of the product. Then the cyclonucleoside was treated with H_2S in dry pyridine to give 5-thioguanosine derivative 11. The structures of the synthesized compounds were confirmed by analytical and spectroscopic analysis.

Antimicrobial activity
The cup-plate technique was used for the determination of the antimicrobial activity. The samples of compounds 2,3,4,5,6,9 and 11 were dissolved in dimethyl formamide (20% concentration). 0.1 mL of each sample was used with some gram-positive bacteria namely, *Sarcina lutea*, *Staphylococcus Aureus* and *Bacillus subtilis* and gram-negative bacteria such as...
Escherichia coli, Agrobacterium and Erwinia sp. under aseptic conditions. The medium for cultivation of the test organisms was nutrient agar. Bacteria were incubated at 30°C for 24 hr and the diameters of the inhibition zones were measured. The results obtained were summarized in Table I.

Experimental Section

All melting points are uncorrected. IR (KBr, cm⁻¹) spectra were recorded on a pye Unicam spectrophotometer and ¹H NMR spectra in DMSO on Germenti 2000 (200 MHz) spectrophotometer using TMS as an internal standard (chemical shifts in δ, ppm). Micro
Table 1 — Antimicrobial activity of the compounds 2-6, 9 and 11

<table>
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<th>Compd</th>
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<th>S. aureus</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>Argobacterium</th>
<th>Erwinia</th>
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Analytical data were performed at the University of Regensburg-Germany.

**Synthesis of 8-diphenylaminoguanosine 2.** A mixture of 8-bromoguanosine (0.5 g, 1.38 mmol) and diphenylamine (0.25 g, 1.39 mmol) in dry DMF (20 mL) was stirred under reflux for 3 hr. The solvent was evaporated under reduced pressure and the residue was washed five times with methanol, then recrystallized from chloroform-methanol (9:1 v/v) to give 2, yield 0.5 g (79%), mp 367°C, Rf 0.42 (CHCl3 : MeOH: 9:1); MS: m/z 450 (M+); 'H NMR: 9.12 (s, 1H, NH ring), 7.21 (s, 2H, NH2), 7.52 (m, 10H, 2ArH), 6.12 (d, J=1.8 Hz, 1H, H-2), 5.35 (dd, J=1.5, 2.6 Hz, 1H, H-3), 5.50 (d, J=6.8 Hz, 1H, 2-0H), 5.25 (d, J=6.7 Hz, 1H, 3-0H), 5.9 (dd, J=1.4, 3.3 Hz, 1H, 1H-3), 4.45 (m, 1H, H-4), 3.7 (m, 2H, H-5); IR (KBr): 3510 (OH), 3275 (NH), 2930 (CH), 1735 (C=O amide). Anal. Calcd for C22H22N6O5: C, 58.66; H, 4.88; N, 17.77. Found: C, 56.8; H, 4.95, N, 25.42%.

**Synthesis of 8-ethylcarboxyhydrazinoguanosine 3.** 8-Aminoguanosine 2 (0.25 g, 1.39 mmol) was added to a mixture of potassium carbonate (0.17 g) and serine (0.176 g, 1.6 mmol) in water (15 mL) and DMF (15 mL). The reaction mixture was stirred under reflux for 5 hr. The solution was evaporated to dryness under reduced pressure, and the residue was dissolved in a minimal volume of water. The precipitate was filtered and washed with methanol and then with water. The crude product 3 was recrystallized from water to give 3, yield 0.217 g (76%), mp 185°C, Rf 0.39 (CHCl3:MeOH: 9:1); MS: m/z 386 (M+); 'H NMR: 9.12 (s, 1H, NH), 7.21 (s, 1H, NH2), 6.81 (s, 1H, NH), 6.12 (d, J=1.8 Hz, 1H, H-1), 5.90 (d, J=1.5, 3.3 Hz, 1H, H-3), 5.55 (d, J=6.8 Hz, 1H, 2-0H), 5.35 (dd, J=1.5, 2.6 Hz, 1H, H-2), 5.25 (d, J=6.8 Hz, 1H, 3-0H) 5.20 (m, 1H, OH), 4.50 (s, 1H, CH); 4.45 (m, 1H, H-4), 3.70 (m, 2H, H-5), 3.54 (m, 2H, CH2); IR (KBr): 3510(OH), 3275(NH), 1670 (C-O amide), 1700 (C=O acid). Anal. Calcd for C21H21N5O6: C, 40.4; H, 4.40; N, 21.7. Found: C, 40.38; H, 4.31; N, 25.42%.

**Synthesis of 8-(2-carboxycyclobutyl)aminoguanosine 4.** 8-Bromoguanosine (0.362 g, 1 mmol) was added to a mixture of potassium carbonate (0.17 g) and serine (0.176 g, 1.6 mmol) in water (15 mL) and DMF (15 mL). The reaction mixture was stirred under reflux for 5 hr. The solution was evaporated to dryness under reduced pressure, and the residue was dissolved in a minimal volume of water. The precipitate was filtered and washed with methanol and then with water. The crude product 4 was recrystallized from water to give 4, yield 0.297 g (76%), mp 185°C, Rf 0.39 (CHCl3:MeOH: 9:1); MS: m/z 386 (M+); 'H NMR: 9.12 (s, 1H, NH), 7.21 (s, 1H, NH2), 6.81 (s, 1H, NH), 6.12 (d, J=1.8 Hz, 1H, H-1), 5.90 (d, J=1.5, 3.3 Hz, 1H, H-3), 5.55 (d, J=6.8 Hz, 1H, 2-0H), 5.35 (dd, J=1.5, 2.6 Hz, 1H, H-2), 5.25 (d, J=6.8 Hz, 1H, 3-0H) 5.20 (m, 1H, OH), 4.50 (s, 1H, CH); 4.45 (m, 1H, H-4), 3.70 (m, 2H, H-5), 3.54 (m, 2H, CH2); IR (KBr): 3510(OH), 3275(NH), 1670 (C-O amide), 1700 (C=O acid). Anal. Calcd for C21H21N5O6: C, 40.4; H, 4.40; N, 21.7. Found: C, 40.38; H, 4.31; N, 25.42%.
Isopropylidene-8-(N,N-dimethylamino)guanosine 6. 8-Bromoguanosine (0.362 g, 1 mmol) was added to a solution of potassium carbonate (0.17 g), glycine (0.15 g, 2 mmol) in 10 mL of water and 10 mL of DMF. The mixture was stirred under reflux for 4 hr and the progress of the reaction mixture was monitored by TLC. The solvent was evaporated to dryness under reduced pressure and was dissolved in a minimal volume of water and the pH of the solution was adjusted to 4 by addition of drops of formic acid. The precipitate obtained was then filtered off and washed with methanol and crystallized from chloroform to give 6, yield 0.271 g (75%), mp 176°C, Rf 0.6, (CHCl₃:MeOH; 9:1); MS: m/z 356 (M⁺); ¹H NMR: 9.00 (s, 1H, NH ring), 7.17 (s, 2H, NH₂), 6.81 (s, 1H, NH), 6.12 (d, J=1.8 Hz, 1H, H-1), 5.35 (dd, J=1.5, 2.6 Hz, 1H, H-2), 5.55 (d, J=6.8 Hz, 1H, 2-0H), 5.90 (dd, J=1.5, 3.3 Hz, 1H, H-3), 4.45 (m, 2H, H-5); IR (KBr): 3510 (OH), 3260 (NH), 1660 (C=O amide). Anal. Calcd for C₉H₁₆N₆O₇; C, 38.81; H, 3.98; N, 17.41. Found: C, 38.73; H, 3.95; N, 17.45%.

2,3-O-Isopropylidene-8-(N,N-dimethylamino)guanosine 9. A solution of 8 (5 mmol) in dry DMF (10 mL) was added dropwise with stirring to a solution of dimethylamine (5 mmol) in dry DMF (20 mL) containing triethylamine (1.7 mL, 13 mmol). The reaction mixture was then stirred continuously for 8 hr at room temperature. The precipitate was filtered off and the filtrate was concentrated to dryness under reduced pressure. The crude product obtained was crystallized from methanol to give 9, yield 81%, m.p. 213°C. MS: m/z 366 (M⁺); ¹H NMR: 9.18 (s, 1H, NH ring), 7.23 (s, 2H, NH₂), 6.00 (d, J=1.8 Hz, 1H, H-1), 5.35 (dd, J=1.5, 2.6 Hz, 1H, H-2), 5.50 (dd, J=1.5, 3.3 Hz, 1H, H-3), 4.45 (m, 1H, H-4), 3.70 (m, 2H, H-5), 4.15 (s, 6H, -N(CH₃)₂), 1.30 (s, 6H, isopropylidene gp); IR (KBr): 3510 (OH), 3250(NH), 1655 (C=O amide). Anal. Calcd for C₁₃H₂₀N₆O₄; C, 49.18; H, 6.01; N, 22.95. Found: C, 49.23; H, 6.02; N, 22.91%.

2,3-O-Isopropylidene-2,5-anhydro-8-(N,N-dimethylamino)guanosine 10. To the Stirred solution of 9 (2 mmol), triphenylphosphine (786 g, 2 mmoles) and triethylamine (1.7 mmol) in dry dichloromethane (5 mL) was added dropwise with stirring (303 g, 3 mmol) in anhydrous dioxane (5 mL), diethylazadicarb oxylate (522 g, 3 mmoles) in anhydrous dioxane (4 mL) was added dropwise. The reaction mixture was stirred for 3 hr and water (1 mL) was added. The solvent was evaporated and the oily residue was extracted with chloroform (2x70 mL). The extract was washed with water and dried with anhyd. magnesium sulphate. The solvent was evaporated and the residue was chromatographed on silica gel column using chloroform-acetone (1:1 to 1:3 v/v) as an eluant to give 10 in 80% yield. MS: m/z 348 (M⁺); ¹H NMR: 9.12 (s, 1H, NH ring), 7.22 (s, 1H, NH), 6.12 (d, J=1.8 Hz, 1H, H-1), 5.32 (dd, J=1.5, 2.6 Hz, 1H, H-2), 5.44 (d, J=6.4 Hz, 1H, 2-0H), 5.21 (d, J=6.7 Hz, 1H, 3-0H), 5.09 (dd, J=1.5, 3.3 Hz, 1H, H-3), 4.45 (m, 1H, H-4), 3.70 (m, 2H, H-5); IR (KBr): 3510 (OH), 3280 (NH), 1670 (C=O amide). Anal. Calcd for C₁₅H₂₆N₆O₄Br; C, 51.72; H, 5.75; N, 24.14. Found: C, 51.69; H, 5.7; N, 24.18%.
chromatographed on silica gel column with chloroform-acetone as an eluant (4:1v/v) to give 11 in 50% yield, m.p. 187°C; MS: m/z 398 (M+); 1H NMR: 9.10 (s, 1H, NH ring), 7.11 (s, 2H, NH2), 6.10 (d, J=1.9 Hz, 1H, H-1), 5.35 (dd, J= 1.5, 2.6 Hz, 1H, H-2), 5.90 (dd, J= 1.5, 3.3 Hz, 1H, H-3), 4.43 (m, 1H, H-4), 3.70 (m, 2H, H-5), 4.25 (s, 6H, N(CH3)2), 3.25 (s, 1H, SH), 1.32 (s, 6H, isopropylidene g p). Anal. Calcd for C15H22N6O4S: C, 47.12; H, 5.76; N, 21.99. Found: C, 47.09; H, 5.71; N, 21.92%.

References