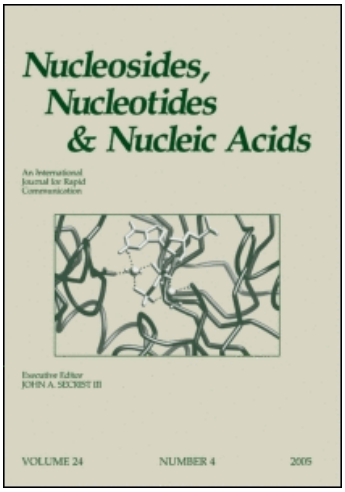

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A NEW SYNTHESIS OF 7-METHYL-8-OXOGUANOSINE

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ABSTRACT: A new, facile synthesis of 7-methyl-8-oxoguanosine is reported. 2-Chloro-7-methylpurine-6,8-dione (5) was silylated with hexamethyldisilazane and the silylated intermediate, 6, glycosylated with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose to yield 2-chloro-7-methyl-9-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl)purin-6,8-dione (8). Deprotection of 8 with sodium hydroxide in aqueous methanol gave 2-chloro-7-methyl-9-(β-D-ribofuranosyl)purine-6,8-dione (9), which was aminated with liquid ammonia or methanolic ammonia to yield 7-methyl-8-oxoguanosine (3).

The role of the human immune system in maintaining health is well established.[1] The recent successes in the isolation of natural immunomodulators and the synthesis of novel compounds which modulate immune systems have opened up new avenues in chemotherapy. Peptides, such as bestatin,[2] and tuftsin,[3] and the thymic hormone[4,5] glycoproteins, such as interferon,[6,7] have been shown to possess immunomodulating properties. Heterocycles, such as levamisole[8] and its purine counterpart, 1,2-dihydrothiazolo[2,3-i]purin-5(6H)-one (NPT-16416)[9], nucleotide peptides[10] from dialyzable leukocyte extracts (DLE), nucleotides such as poly A-U and poly I-C[9] and nucleosides have also been shown to be effective modulators of the immune system.

Nucleoside formulations containing hypoxanthine (inosine), such as methisoprinol[11] and 1,9-dihydro-9-[1-(1-hydroxyethyl)heptyl]-6H-purin-6-one (NPR 15392)[12] have been shown to increase cell-mediated immune

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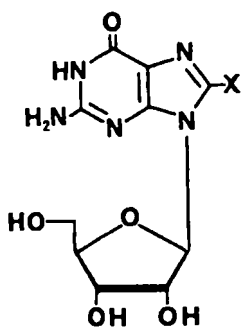
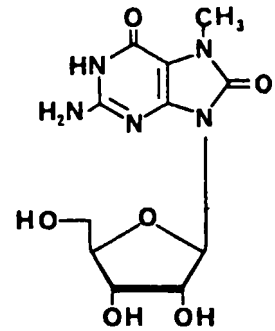
functions in vitro and in vivo. We have prepared[13,14] a number of guanosine derivatives which have been evaluated as modulators of the immune response by Goodman and Wiegle. Of these derivatives, 8-bromoguanosine (1), 8-mercaptoguanosine (2), and 7-methyl-8-oxoguanosine (3) have been reported by these investigators[15-17] as stimulators of the humoral, B-cell, immune system. The enhanced potency of 3 as an



1 X,Br; 2 X,SH

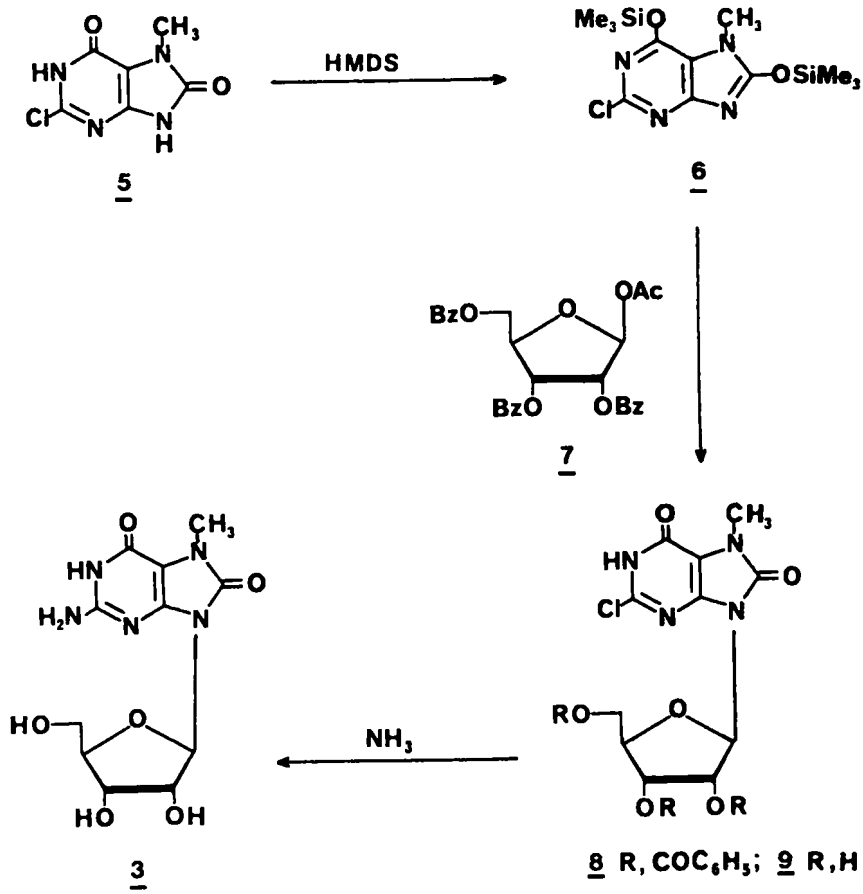


3

immunostimulant[18] prompted us to develop an alternate synthesis for the preparation of 3. Our previously reported method[14] involved seven steps and gave a 9% overall yield of 7-methyl-8-oxoguanosine (3). The method herein reported provides 3 in a 28-44% overall yield from 2-chloro-7-methylpurin-6,8-dione (5).

Theobromine (4) was converted[19] to 2-chloro-7-methylpurin-6,8-dione (5) by the procedure of Borowitz et al.[20] After being dried over phosphorous pentoxide, 5 was silylated with hexamethyldisilazane using ammonium sulfate as the catalyst. The excess hexamethyldisilazane was removed by evaporation in vacuo and the hygroscopic residue, 6, was used without further purification. The silylated heterocycle, 6, was glycosylated with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (7) in dry acetonitrile with trimethylsilyl triflate as the catalyst[21,22] to give 2-chloro-7-methyl-9-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl)purin-6,8-dione (8) in 87.5% yield. Removal of the ester functions was accomplished in 87% yield with sodium hydroxide in aqueous methanol to provide 2-chloro-7-methyl-9-(β-D-ribofuranosyl)purin-6,8-dione (9) after acidification and extraction of the benzoic acid. Compound 9 was also obtained

in 62.5% yield from the crude glycosylation mixture by deprotection as described above. The chlorine atom at position 2 in compound 9 was displaced with liquid ammonia in a steel bomb at 150°C to give 3 in 43% yield, while amination of 9 with methanolic ammonia at 150°C led to 3 in 60% yield.



EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined at 90 MHz with a JEOL FX-90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of H_2O as indicated by elemental analyses was verified by ^{1}H NMR. Optical rotations were recorded on a Perkin-Elmer 241 automatic polarimeter.

Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was run on aluminum backed silica gel 60 F-254 (EM Reagents) plates. Preparative scale chromatography was conducted using flash chromatography techniques. J. T. Baker silica gel (~40 μm) or Kiesel gel 60 EM (40-63 μm) was used for flash chromatography. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 35°C.

2-Chloro-7-methyl-9-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl)purin-6,8-dione (8)

A suspension of 2-chloro-7-methylpurin-6,8-dione (5) (22.3 g, 111.2 mmol)[20] and ammonium sulfate (0.9 g, 6.8 mmol) in hexamethyldisilazane (300 mL) was refluxed for 12 h. The solvent was evaporated in vacuo and the residue dissolved in dry acetonitrile (1250 mL). 1-O-Acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (7) (49.2 g, 97.5 mmol) and trimethylsilyl triflate (23.9 mL) were added and the solution refluxed overnight. The solvent was evaporated in vacuo and the residue dissolved in ethyl acetate (500 mL). The organic extract was washed with sat. aq. $NaHCO_3$ (2 x 250 mL), water (2 x 200 mL) and sat. aq. NaCl (150 mL) in succession. After drying over anhydrous Na_2SO_4, the solvent was evaporated in vacuo and the resulting oil chromatographed over silica gel with 50% acetone in hexane as elutant to yield pure 8 (52.91 g, 84.7%) as an oil. Attempted crystallization from water converted the oil into an amorphous solid which melted above 80°C: $[\alpha]_D^{22}$ -39.3 (C 3.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.6 (s, 3), 4.76 (bs, 3), 5.2-5.6 (m, 3), 6.2-7.2 (m, 15). TLC (20% ethyl acetate in benzene) Rf 0.61. Anal. Calcd. for $C_{32}H_{25}O_9N_4Cl \cdot H_2O$:C, 57.96; H, 4.07; N, 8.45; Cl, 5.36. Found: C, 58.12; H, 3.98; N, 8.12; Cl, 5.40.

2-Chloro-7-methyl-9-(β-D-ribofuranosyl)purine-6,8-dione (9)

Method A: To a solution of 2-chloro-7-methyl-9-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl)purin-6,8-dione (8) (11.78 g, 18.4 mmol) in methanol (300 mL) was added aqueous 1 N sodium hydroxide (60.1 mL) and stirred at room temperature for 6 h. Solvent was removed in vacuo and the residue dissolved in water (200 mL). After acidification to pH 1-2 with aqueous 2N hydrochloric acid, the solution was washed with ether (2

x 200 mL) and the aqueous phase cooled, upon which 6 crystallized out as an off-white solid (5.3 g, 87%), mp 154-157°C: $[\alpha]_D^{22}$ -30.3 (C 1, CH_3OH); ^1H NMR (Me_2SO d_6) δ 3.48 (s, 3), 3.63 (d, 1), 3.9 (m, 1), 4.2 (t, 1), 5.48 (t, 1), 5.7 (d, 1). TLC (30% CH_3OH in ethyl acetate) Rf 0.37. Anal. Calcd. for $C_{11}H_{13}N_4O_6Cl$: C, 39.7; H, 3.91; N, 16.84; Cl, 10.68. Found: C, 39.43; H, 3.99; N, 16.58; Cl, 10.80.

Method B: A suspension of 2-chloro-7-methylpurin-6,8-dione(5) (12.08 g, 60.2 mmol) and ammonium sulfate (0.5 g, 3.8 mmol) in hexamethyldisilazane (165 mL) was refluxed for 12 h. The solvent was evaporated in vacuo and the residue dissolved in dry acetonitrile (680 mL). 1-O-Acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (7) (26.7 g, 52.9 mmol) and trimethylsilyl triflate (13 mL) were added and the solution refluxed overnight. After workup as previously described in synthesis of 8, the residue was dissolved in methanol (1000 mL), 1 N aqueous sodium hydroxide (200 mL) added and the solution stirred at room temperature for 6 h. Solvent was evaporated in vacuo and the residue dissolved in water (175 mL). After acidification to pH 1-2 with aqueous 2N HCl, the acidic aqueous solution was washed with ether (3 x 500 mL). Cooling the aqueous solution resulted in crystallization of 9 as an off white crystalline solid (11.0 g, 62.5%) identical in all respects to the material obtained by Method A.

7-Methyl-8-oxoguanosine (3)

Method A: To a suspension of 2-chloro-7-methyl-9-(β-D-ribofuranosyl)-purin-6,8-dione (9) (2.12 g, 6.4 mmol) in methanol (10 ml), cooled in a dry ice-acetone bath in a 125 mL stainless steel bomb was added liquid ammonia (50 mL). The bomb was sealed and heated in an oil bath at 150°C for 5 h. The bomb was allowed to cool overnight at room temperature and opened after cooling in a dry ice-acetone bath. After evaporation of the ammonia at room temperature, the residue was chromatographed over silica gel with 20% methanol in chloroform followed by 30% methanol in chloroform as the elutant to yield 1.18 g (60%) of 3. This product was identical in all respects to an authentic sample [14].

Method B: To 2-chloro-7-methyl-9-(β-D-ribofuranosyl)purin-6,8-dione (9) (0.97 g, 2.5 mmol) in a steel bomb was added liquid ammonia (25 mL)

after which the bomb was sealed and heated to 150°C for 8 h. The bomb was allowed to cool overnight, then cooled in a dry ice-acetone bath and opened. After evaporation of the ammonia at room temperature, the residue was dissolved in warm 50% aqueous methanol (50 mL), decolorised with Norit A decolorising carbon, filtered, and the solvent evaporated in vacuo to yield a white solid which was homogeneous on TLC (30% CH_3OH in chloroform). This solid was crystallized from 10% aqueous methanol (70 mL) to yield 0.40 g (44%) of 3 which was identical in all respects to an authentic sample.[14]

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