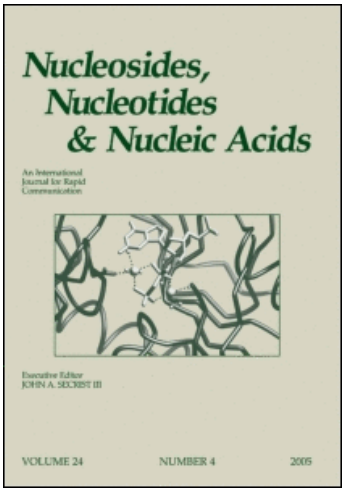

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Facile Method for the Preparation of 7-Methyl-8-oxoguanosines as an Immunomodulator

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FACILE METHOD FOR THE PREPARATION OF 7-METHYL-8-OXOGUANOSINES AS AN IMMUNOMODULATOR¶

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ABSTRACT: Reaction of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium iodide (**2a**) with hydrogen peroxide in acetic acid gave the corresponding 7-methyl-8-oxoguanosine derivative (**3a**) in good yield. Deprotection of **3a** easily gave 7-methyl-8-oxoguanosine (**1**), which is well-known as an immunomodulator. Substitution of acetyl group at the N^2-position of guanine ring accelerated the oxidation reaction of the 7-methylguaninium iodide.

INTRODUCTION

The need for adjuvant therapy to boost the immune system of AIDS and cancer patients has brought this area of immunopotentiation into sharp focus. Many different types of compounds (i.e., glycoproteins, peptides, nucleosides, and small heterocycles) have been demonstrated to possess immunostimulatory properties.[1] Among nucleosides, 7-methyl-8-oxoguanosine (**1**)[2] has been evaluated as a modulator of B-cell activation.[3] Recently, we have reported that 7-methyl-8-oxoguanosine derivative is prepared by the direct oxygenation of 7-methylguaninium iodide *via* a photoinduced

¶ This paper is dedicated to the late Professor Tsujiaki Hata.

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electron transfer.[4] In spite of its important biological activities, conventional methods[2,4] for the preparation of **1** involved some difficulties with respect to the yield. These difficulties prompted us to discover a convenient method producing **1** in good yield.

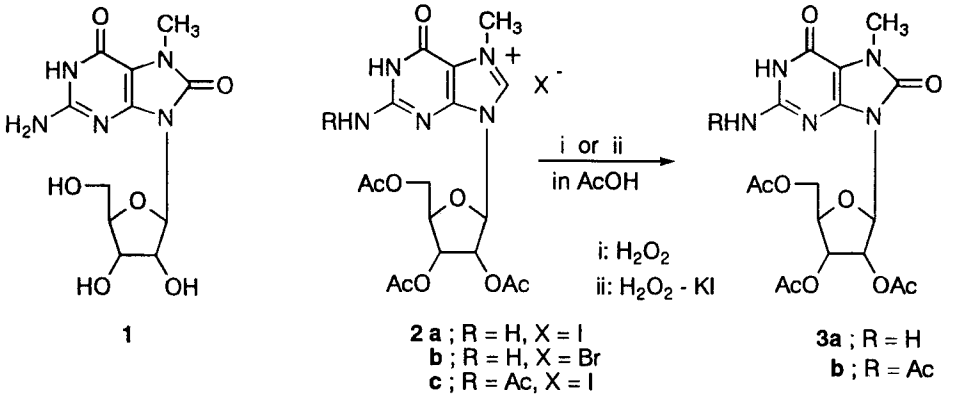
This paper describes a facile method for the preparation of 7-methyl-8-oxoguanosines by oxidation of 7-methylguaninium halides with hydrogen peroxide.

RESULTS AND DISCUSSION

Starting materials, 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium halides (**2a**, 68% and **2b**, 62%) and N^2-acetyl-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium iodide (**2c**, 55%), were easily obtained by the reaction of 2',3',5'-triacetylguanosine or N^2,2',3',5' -tetraacetylguanosine with methyl iodide or methyl bromide in DMF according to the method described in the literature.[5] Reaction of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium iodide (**2a**) with H_2O_2 (2 eq.) at 37 ℃ for 20 h gave 2',3',5'-tri-*O*-acetyl-7-methyl-8-oxoguanosine (**3a**) in 67 % yield. Under the reaction of **2a** with H_2O_2 in the dark, the formation of **3a** was similarly observed on the basis of TLC analyses. Deprotection of **3a** with methanolic ammonia smoothly afforded 7-methyl-8-oxoguanosine (**1**) in 82% yield. The structure of **1** was supported by spectral data (^{1}H NMR, ^{13}C NMR, and Mass) and microanalytical results.

On the other hand, analogous treatment of the guaninium bromide (**2b**) with H_2O_2 (2 eq.) did not give the oxidized product (**3a**). However, the addition of KI (2 eq.) to the reaction mixture of **2b** resulted in the formation of **3a** in 54% yield. The use of **2b** as a starting material prolonged the reaction time (65 h) and decreased the yield of **3a**. The presence of iodide anion in the reaction mixture is a prerequisite for the formation of **3a**. Although H_2O_2-KI oxidation system would generate some active oxygen, the detailed mechanism of this reaction is not clear at present.

N^2-Acetyl-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium iodide (**2c**) by the reaction with H_2O_2 at 37 ℃ for 5 h was smoothly converted into the corresponding N^2-acetyl-7-methyl-8-oxoguanosine (**3b**) in 71% yield. Substitution of acetyl group at the N^2-position of guanine ring improved the isolation yield and shortened the reaction time. Figure 1 shows TLC analyses of the formation of **3a** and **3b** in the reaction of **2a** and **2c** with H_2O_2 as a function of reaction time. It is clear that the rate of the formation of 7-methyl-8-oxoguanosines under the present oxidation is in the order of **3b** > **3a**.



1

2a ; R = H, X = I
b ; R = H, X = Br
c ; R = Ac, X = I

3a ; R = H
b ; R = Ac

SCHEME 1

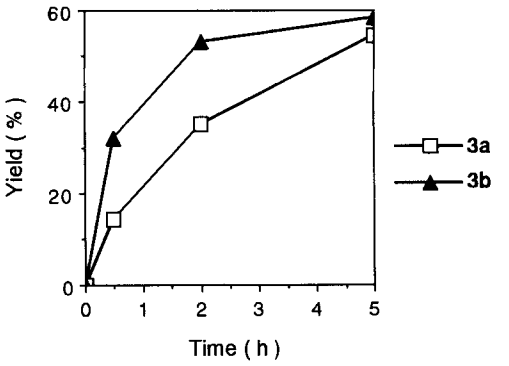


FIG. 1. TLC analyses of the formation of 3a and 3b

This acceleration effect of the N^2-acetyl group can be explained in terms of nucleophilic attack of an active oxygen generated from H_2O_2-KI at the C_8-position of guanine ring. Because substitution of the acetyl group at the N^2-position affects the chemical shift of the C_8-proton [from 9.44 ppm (**2a**) to 9.65 ppm (**2c**)], the C_8 of **2c** should be more easily attacked by an active oxygen than that of **2a**.

In this report, we present a convenient method for the preparation of 7-methyl-8-oxoguanosine as an immunomodulator *via* the oxidative reaction of 7-methyl-9-ribofuranosylguaninium halides (**2**). The results involve interesting implications for both the oxidative damage of nucleic acids[6] and a significant increase of 7-methyl-8-oxoguanine in the urine of patients with leukemia.[7]

EXPERIMENTAL SECTION

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analyses were carried out at the microanalytical laboratory of Gifu Pharmaceutical University. All UV measurements were carried out with a Hitachi U-2001 spectrophotometer. ^{1}H-NMR spectra were recorded at 400 MHz on a JEOL α-400 spectrometer. Chemical shifts are quoted in parts per million (s = singlet, d = doublet, dd = double doublet, t = triplet, and m = multiplet). Mass spectra (MS) were measured at 70 eV with a JEOL JMS-D300 spectrometer and a Shimadzu QP 1000A. TLC for the assay of guanosines on Silica gel 60 plates (Merck, art 5715) using chloroform-methanol (5:1) as an eluent and TLC-scanning was carried out with Shimadzu CS-9000 dual-wavelength flying-spot scanner (detection, the corresponding maximum peak of guanosines). Column chromatography was carried out on a silica gel (Wako gel C-300).

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium Iodide (2a). A mixture of 2',3',5'-triacetylguanosine (4.09 g, 10 mmole) and methyl iodide (6.3 ml, 100 mmole) in DMF (20 ml) was stirred at 37 ℃ for 18 h. The solvent was removed under reduced pressure and the residue was dissolved in acetone. The resulting precipitate was collected by filtration. Recrystallization from ethanol gave an analytically pure **2a** (3.765 g, 68%); m.p. 193-194 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) : δ 2.03 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 4.01 (3H, s, N_7-CH_3), 4.29 (1H, dd, J = 6.0, 12.1 Hz, 5'H), 4.37 (1H, dd, J = 3.4, 12.2 Hz, 5'H), 4.45 (1H, m, 4'H), 5.53 (1H, t, J = 5.9 Hz, 3'H), 5.73 (1H, t, J = 5.0 Hz, 2'H), 6.17 (1H, d, J = 4.2 Hz, 1'H), 7.21 (2H, br s, C_2-NH_2), 9.35 (1H, s, C_8-H), 11.76 (1H, s, N_1-H). ^{13}C-NMR (DMSO-d_6, 100 MHz) : δ 20.27, 20.32, 20.60, 35.81, 63.01, 69.72, 72.90, 80.36, 87.10, 107.71, 136.83, 148.90, 153.31, 155.59, 169.14, 169.23, 170.10. MS *m/z*: 423 (M^+-HI). Anal. Calcd. for $C_{17}H_{22}N_5O_8I$: C, 37.04; H, 4.02; N, 12.70: Found C, 37.00; H, 4.05; N, 12.89.

9-(2, 3, 5-Tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium Bromide (2b). A mixture of 2',3',5'-triacetylguanosine (10.225 g, 25 mmole) and methyl bromide (25 ml, 250 mmole) in DMF (100 ml) was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in acetone. The resulting precipitate was collected by filtration. Recrystallization from ethanol gave an analytically pure **2b** (7.826 g, 62%); m.p. 208-209 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) : δ 2.03 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 4.01 (3H, s, N_7-CH_3), 4.30 (1H, dd, J = 6.0, 12.1 Hz, 5'H), 4.38 (1H, dd, J = 3.4, 12.2 Hz, 5'H), 4.45 (1H, m, 4'H), 5.53 (1H, t, J = 5.9 Hz, 3'H), 5.73 (1H, t, J = 5.0 Hz, 2'H), 6.18 (1H, d, J = 3.9 Hz, 1'H), 7.33 (2H, br s, C_2-NH_2), 9.44 (1H, s, C_8-H), 11.78 (1H, s, N_1-H). ^{13}C-

NMR (DMSO-d_6, 100 MHz) : δ 20.24, 20.31, 20.59, 35.77, 63.01, 69.73, 72.91, 80.38, 87.07, 107.70, 136.84, 148.85, 153.17, 155.67,169.14, 169.23, 170.10. MS *m/z*: 423 (M^+-HBr). Anal. Calcd. for $C_{17}H_{22}N_5O_8Br$: C, 40.49; H, 4.40; N, 13.89: Found C, 40.30; H, 4.38; N, 13.99.

N^2-Acetyl-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-7-methylguaninium Iodide (2c). A mixture of N^2,2',3',5'-tetraacetylguanosine[8] (1.507 g, 3.34 mmole) and methyl iodide (2.1 ml, 33.4 mmole) in DMF (10 ml) was stirred at 37 ℃ for 24 h. The solvent was removed under reduced pressure and the residue was dissolved in toluene. The resulting precipitate was collected by filtration. Recrystallization from ethanol gave an analytically pure **2c** (1.095 g, 55%); m.p. 186-190 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) : δ 2.05 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 2.24 (3H, s, $COCH_3$), 4.11 (3H, s, N_7-CH_3), 4.32 (1H, dd, *J* = 6.1, 12.4 Hz, 5'H), 4.39 (1H, dd, *J* = 2.4, 12.3 Hz, 5'H), 4.51 (1H, q, *J* = 4.4 Hz, 4'H), 5.52 (1H, t, *J* = 5.6 Hz, 3'H), 5.73 (1H, t, *J* = 5.6 Hz, 2'H), 6.28 (1H, d, *J* = 4.4 Hz, 1'H), 9.65 (1H, s, C_8-H), 12.03 (1H, s, C_2-NHAc), 12.64 (1H, s, N_1-H). ^{13}C-NMR (DMSO-d_6, 100 MHz) : δ 20.24, 20.31, 20.60, 24.07, 36.09, 62.99, 69.55, 73.09, 80.50, 87.23, 111.49, 138.93, 146.44, 150.64, 151.31, 169.07, 169.19, 170.06, 174.10. MS *m/z*: 465 (M^+-HI). Anal. Calcd. for $C_{19}H_{24}N_5O_9I$: C, 38.46 ; H, 4.08 ; N, 11.80: Found C, 38.21; H, 3.99; N, 11.62.

2',3',5'-Triacetyl-7-methyl-8-oxoguanosine (3a). A mixture of **2a** (1.79 g, 3.25 mmole) and 30% hydrogen peroxide (0.50 ml, 6.5 mmole) in acetic acid (30 ml) was stirred at 37 ℃ for 20 h. The excess hydrogen peroxide was quenched by 1 N sodium sulfite solution. The mixture was evaporated under reduced pressure and the residue was dissolved in water (30 ml). The solution was extracted by chloroform and the extract was dried over sodium sulfate. The solvent was evaporated under reduced pressure and the resulting residue was chromatographed on a silica gel column with chloroform-methanol (50:1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **3a** (0.9553 g, 67%); m.p. 185-187 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) : δ 2.00 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 3.32 (3H, s, N_7-CH_3), 4.10 (1H, dd, *J* = 6.0 and 11.6 Hz, 5'H), 4.20 (1H, m, 4'H), 4.35 (1H, dd, *J* = 3.8, 11.8 Hz, 5'H), 5.54 (1H, t, *J* = 6.0 Hz, 3'H), 5.75 (1H, d, *J* = 4.4 Hz, 1'H), 5.98 (1H, t, *J* = 5.4 Hz, 2'H), 6.54 (2H, br s, C_2-NH_2), 10.93 (1H, s, N_1-H). ^{13}C-NMR (DMSO-d_6, 100 MHz) : δ 20.27, 20.31, 20.49, 28.28, 62.87, 70.15, 70.66, 78.65, 99.44, 147.75, 150.37, 151.88, 153.47, 169.36, 169.41, 170.10. MS *m/z*: 439 (M^+). Anal. Calcd. for $C_{17}H_{21}N_5O_9$: C, 46.47; H, 4.82; N, 15.94: Found C, 46.27; H, 4.98; N, 15.21.

Reaction of 2b with H_2O_2-KI. A mixture of **2b** (551 mg, 1 mmole), 30% H_2O_2 (155 μl, 2 mmole), and KI (333 mg, 2 mmole) in acetic acid (15 ml) was stirred at 37 ℃ for 65 h. The excess hydrogen peroxide was quenched by 1 N sodium sulfite solution. The mixture was evaporated under reduced pressure and the residue was dissolved in water (30 ml). The solution was extracted by chloroform and the extract was dried over sodium sulfate. The solvent was evaporated under reduced pressure and the resulting residue was chromatographed on a silica gel column with chloroform-methanol (50:1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **3a** (237 mg, 54%), which was identical in every respect with the same sample obtained above.

***N*^{2},2',3',5'-Tetraacetyl-7-methyl-8-oxoguanosine (3b).** A mixture of **2c** (502 mg, 0.846 mmole) and 30% H_2O_2 (131 μl, 1.69 mmole) in acetic acid (15 ml) was stirred at 37 ℃ for 5 h. The excess H_2O_2 was quenched by 1 N sodium sulfite solution (3.5 ml). The mixture was evaporated under reduced pressure and the residue was dissolved in water (30 ml). The solution was extracted by chloroform and the extract was dried over sodium sulfate. The solvent was evaporated under reduced pressure and the resulting residue was chromatographed on a silica gel column with chloroform. The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **3b** (289 mg, 71%); m.p. 99-102.5 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz) : δ 2.06 (6H, s, $COCH_3$ x 2), 2.12 (3H, s, $COCH_3$), 2.29 (3H, s, $COCH_3$), 3.56 (3H, s, N_7-CH_3), 4.38 (2H, m, 4'H, 5'H), 4.69 (1H, dd, J = 5.1, 11.0 Hz, 5'H), 5.75 (1H, t, J = 4.9 Hz, 3'H), 5.91 (1H, t, J = 5.4 Hz, 2'H), 6.09 (1H, d, J = 4.9 Hz, 1'H), 9.54 (1H, s, C_2-NHAc), 11.97 (1H, s, N_1-H). ^{13}C-NMR (DMSO-d_6, 100 MHz) : δ 17.95, 20.13, 20.47, 23.73, 28.62, 57.82, 63.07, 70.41, 71.59, 78.59, 84.08, 105.21, 142.90, 146.60, 150.04, 150.97, 169.42, 169.55, 171.00. MS *m/z*: 481 (M^+). Anal. Calcd. for $C_{19}H_{23}N_5O_{10}$ · 1/2H_2O: C, 46.53; H, 4.93; N, 14.28: Found C, 46.79; H, 4.91; N, 13.88.

Deprotection of 3a with Methanolic Ammonia. A mixture of **3a** (639 mg, 1.45 mmole) in methanolic ammonia (10 ml) was stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The residue was dissolved into methanol and the resulting precipitate was collected by filtration. Recrystallization from water gave an analytically pure **1** (374 mg, 82%); m.p. 264-265 ℃ (decomp.). ^{1}H NMR (DMSO-d_6, 400 MHz) d 3.35 (3H, s, N_7-CH_3), 3.42 (1H, dd, J = 5.1, 11.9 Hz, 5'H), 3.56 (1H, dd, J = 4.4, 11.7 Hz, 5'H), 3.77 (1H, m, 4'H), 4.07 (1H, t, J = 6.0 Hz, 3'H), 4.79 (1H, br s, 5'OH), 4.82 (1H, t, J = 6.4 Hz, 2'H), 4.94 (1H, br s, 3'OH), 5.23 (1H, br s, 2'OH), 5.58 (1H, d, J = 6.4 Hz, 1'H), 6.47 (2H, s, C_2-NH_2), and 10.87 (1H, s, N_1-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 28.23, 62.29, 69.85, 70.80, 85.05,

85.70, 99.36, 146.48, 151.02, 151.82, 153.22. MS *m/z*: 313 (M^+). Anal. Calcd. for $C_{11}H_{15}N_5O_6$: C, 42.17; H, 4.83; N, 22.36: Found C, 41.77; H, 4.89; N, 22.13.

TLC Analyses of the Formation of 7-Methyl-8-oxoguanosines (3a or 3b) by the Oxidation with H_2O_2. A mixture of 7-methylguaninium iodides (**2a** or **2c**) (0.02 mmole) and H_2O_2 (3 μl, 0.04 mmole) in acetic acid (1 ml) was stirred at 37 ℃. Formation of the 7-methyl-8-oxoguanosines was followed spectrophotometrically with a TLC scanner and the yield was calculated by $A/A_{100} \times 100$ (A_{100} = the theoretical peak area of 7-methyl-8-oxoguanosine (**3a** or **3c**) in 100% yield, A = the peak area of 7-methyl-8-oxoguanosine (**3a** or **3c**) in the time course of the oxidation).

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