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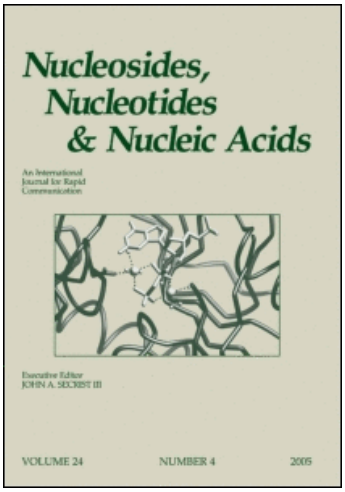

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Note: A Convenient Method for the Preparation of N^2, N^2-Dimethylguanosine

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NOTE

A CONVENIENT METHOD FOR THE PREPARATION OF N^2,N^2-DIMETHYLGUANOSINE

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Abstract: The preparation of N^2,N^2-dimethylguanosine is described. The use of the 2-(*p*-nitrophenyl)ethyl group instead of the benzyl protecting group for the O^6 position of the guanine ring resulted in better yields and shorter protocols.

Small nuclear RNAs (snRNAs) such as U1, U2, U4 and U5 have N^2, N^2, 7-trimethylguanosine at the 5'-end which play an important role in splicing of eukaryotic pre-mRNAs[1]. The synthesis of several compounds having this cap structure has been described[2]. The starting material, N^2,N^2-dimethylguanosine, could be prepared from 2-amino-6-chloro-9-β-D-ribofuranosylpurine or from 6-thioguanosine as described by Gerster and Robins[3,4] or from 5-amino-1-β-D-ribofuranosyl-1-imidazolecarboxamide (AICA-riboside)[5]. The route described by Gerster and Robins is the most commonly used and has two main steps a) transforming guanosine derivatives to 2-fluoroinosine and b) displacement of fluoride with dimethylamine[3,4]. Direct methylation of guanosine does not afford the desired N^2,N^2-dimethylguanosine[3], except if the 1,3-benzodithiol-2-yl group is introduced into the 2-amino group[6]. In order to obtain the 2-fluoroinosine derivative, protection of position 6 of guanosine derivatives should be used. For that purpose the benzyl group (which can be removed by hydrogenolysis) is used[3,4]. In the present communication we described the preparation of N^2,N^2-dimethylguanosine starting from guanosine using the 2-(*p*-nitrophenyl)ethyl (Npe) group for the position 6 of guanine.This group, developed by Pfleiderer *et al.* [7,8], is removed by bases through a β-elimination mechanism. The removal conditions are particulary suited to the chemical properties of this product that is obtained in higher yields.

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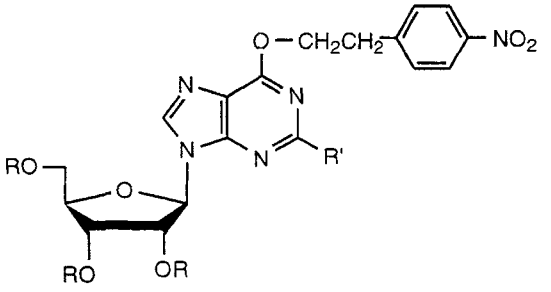
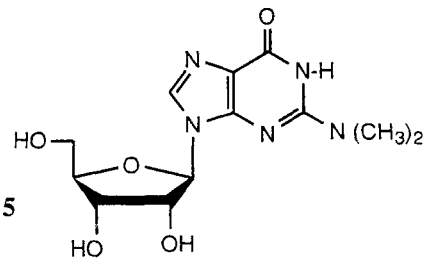
2',3',5'-Tris-O-acetyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**1**) was prepared from guanosine by acetylation with acetic anhydride in pyridine and subsequent Mitsunobu reaction to introduce the Npe group as described in ref. 8. Removal of the acetyl groups of compound **1** was done by treatment with aq. ammonia / MeOH / dioxane[8]. Conversion of Npe-protected guanosines **1** and **2** to Npe-protected 2-fluoroinosines **3** and **4** was performed by diazotation and fluoride displacement with sodium nitrite and tetrafluoroboric acid in acetone / water mixtures at -20 °C[3,4]. After the reaction was done, the reaction mixture was neutralized and compounds **3** and **4** precipitated in quantitative yields immediatly or after the removal of acetone in the rotatory evaporator making easy the separation of the products from the aqueous mixture full of salts. Analytical pure samples could be obtained by silica gel chromatography but the crude products were very clean and were used in the next step without further purification. Treatment of compounds **3** and **4** with a 33% dimethylamine solution in ethanol overnight at 55 °C yield N^2,N^2-dimethylguanosine (**5**) as the only nucleoside product together with the products resulting from the removal of the protecting groups. The desired compound was easily purified by silica gel chromatography. The removal of the acetyl and Npe groups and the displacement of the 2-fluoro group by dimethylamine was also studied at room temperature but the removal of the Npe group was only partial (35%) in an overnight treatment. So the temperature was increased at 55 °C in order to run the reaction to completion.



1 R = acetyl, R' = NH_2
2 R = H, R' = NH_2
3 R = acetyl, R' = F
4 R = H, R' = F



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When the triacetyl derivative **1** was used for the preparation of 2-fluoroinosine derivative **3** higher yields were obtained. If diazonium salt displacement was done with the triacetyl derivative **1**, dimethylguanosine was obtained in a 48 % overall yield. A 21% overall yield was obtained if this reaction was done with the unprotected nucleoside **2**. In conclusion, we have shown that N^2,N^2-dimethylguanosine could be prepared in 6 steps from guanosine using Npe protecting group for the position 6 of guanine instead of the

previously described benzyl group. The base-labile Npe group is more convenient because its removal could be done at the same time as the fluoride displacement by dimethylamine. A similar protocol could be used to prepare other N^2-derivatives of guanosine.

EXPERIMENTAL SECTION

Abbreviations used : DBU : 1,8-diazabicyclo[5.4.0]undec-7-ene, DCM : dichloromethane, EtOH : ethanol, MeOH : methanol, Npe : 2-(4-nitrophenyl)ethyl . 2',3',5'-Tris-O-acetyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**1**) was prepared as described[8]. HPLC conditions : Column : Nucleosil 120C18, 250 x 4 mm, flow rate 1 ml / min, A : 20 mM triethylammonium acetate, B : acetonitrile / water (1:1). A 40 minutes linear gradient from 5 to 95 % B.

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O^6-[2-(4-Nitrophenyl)ethyl]guanosine (2).

Compound **1**[7] (4.0 g, 7.4 mmol) was treated with 20 ml of 10% aq. ammonia / methanol / dioxane (2:1:1) mixture[8] for 16 hr at room temperature and then the solution was evaporated to dryness. The residue was purified on a silica gel column eluted with a 0 to 10 % MeOH gradient in DCM. Yield : 1.6 g (52%). TLC (10% MeOH / DCM) Rf = 0.30. HPLC : retention time (see conditions above) 31.6 min. ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm) : 8.2 (d, 2H, Npe group), 8.1 (s, 1H, H-8), 7.6 (d, 2H, Npe group), 6.46 (broad s, 2H, NH_2), 5.7 (m, 2H, H-1', OH), 5.4 (d, 1H, OH), 5.1 (m, 1H, OH), 4.68 (t, 2H, Npe group), 4.45 (d, 1H, H-2'), 4.11 (m, 1H, H-3'), 3.9 (m, 1H, H-4'), 3.5-3.6 (m, 1H, H-5'), 3.26 (t, 2H, Npe group).

2',3',5'-Tris-O-acetyl-2-fluoro-O^6-[2-(4-Nitrophenyl)ethyl]inosine (3).

Compound **1** (8.4 g, 15.5 mmol) was dissolved in acetone and the solution was cooled at -20°C. To the magnetic stirred solution 70 ml of a 50% aqueous tetrafluoroboric acid solution was added slowly. Then 3.7 g (28.2 mmol) of sodium nitrite dissolved in 10 ml of water was added dropwise. After the addition, the reaction mixture was stirred at -20°C for 30 min and neutralized with a 50% NaOH aqueous solution at -20°C. An oily product precipitated staying on the walls and the aqueous solution was separated. The solid was dried in a diseccator and was used without further purification. An analytical sample was purified by silica gel chromatography for characterization. TLC (10% MeOH / DCM) Rf = 0.5. ^{19}F-NMR (Cl_3CD) δ (ppm) : 27.1 . ^{1}H-NMR (Cl_3CD) δ (ppm) : 8.2 (d, 2H, Npe); 8.1 (s, 1H, H-8); 7.6 (d, 2H, Npe); 6.1 (d, 1H, H-1'); 5.8 (m, 1H, H-2'); 5.6 (m, 1H, H-3'); 4.8 (t, 2H, Npe); 4.5-4.3 (m, 3H, H-4' and H-5'); 3.3 (t, 2H, Npe); 2.1 (s, 6H, CH_3); 2.0 (s, 3H, CH_3).

2-Fluoro-O^6-[2-(4-Nitrophenyl)ethyl]inosine (4).

Compound **2** (1.6 g, 3.8 mmol) dissolved in acetone was treated with 16.3 ml of a 50% tetrafluoroboric acid aqueous solution and 0.46 g (6.6 mmol) of sodium nitrite dissolved in 1.7 ml of water at -20 °C as described for compound **3**. The solution resulting from the neutralization was concentrated until a sticky solid appeared. This solid was separated from the aqueous solution and dried in a diseccator with phosphorous pentoxide and was used without further purification. An analytical sample was purified by silica gel chromatography and characterized as 2-fluoro-O^6-[2-(4-Nitrophenyl)ethyl]inosine (**4**). TLC (10% MeOH / DCM) Rf = 0.36. HPLC (see conditions above) : retention time = 36.0 min. ^{19}F-NMR (DMSO-d_6) : 25.3 ppm. ^{1}H-NMR (DMSO-d_6) : 8.2 (d, 2H, Npe); 8.0 (s, 1H, H-8); 7.6 (d, 2H, Npe); 5.8 (m, 2H, H-1', OH); 5.5 (d, 1H, OH); 5.2 (m, 1H, OH); 4.8 (t, 2H, Npe); 4.5 (d, 1H, H-2'); 4.1 (m, 1H, H-3'); 3.9 (m, 1H, H-4'); 3.5 (m, 2H, H-5'); 3.3 (t, 2H, Npe).

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N^2,N^2-Dimethylguanosine (5).

From compound **3.**

The crude product **3** described above was dissolved in 8 ml of dioxane and 80 ml of a 33% dimethylamine solution in ethanol was added. The resulting mixture was heated for 16 hr at 55 °C. The solution was cooled at room temperature and concentrated to dryness. The product was purified with a silica gel column eluted with a 10 to 35 % MeOH gradient in DCM. Yield : 2.2 g (48 % from compound **1**). TLC (20% / DCM) Rf = 0.12. HPLC (see conditions above) : retention time = 13.2 min. ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm) : 8.0 (s, 1H, H-8); 5.8 (d, 1H, H-1'); 5.4 (d, 1H, OH); 5.1 (d, 1H, OH); 4.5 (d, 1H, H-2'); 4.1 (m, 1H, H-3'); 3.9 (m, 1H, H-4'); 3.5 (m, 2H, H-5'); 2.6 (s, 6H, CH_3).

From compound **4.**

The crude product **4** described above was treated with 20 ml of a 33% dimethylamine solution in ethanol and 8 ml of dioxane for 16 hr at 55°C as decribed above. Yield : 0.45 g (21% from compound **1** and after silica gel purification). Chromatographic data and spectroscopic measurements show that the product obtained from compound **3** is identical to the product obtained from compound **4**.

When product **4** was treated with 33% dimethylamine / ethanol for 16 hr at room temperature instead of at 55°C a 1 : 2 mixture of N^2,N^2-dimethylguanosine (**5**) and N^2,N^2-dimethyl-O^6-[2-(4-nitrophenyl)ethyl)]guanosine was obtained.

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