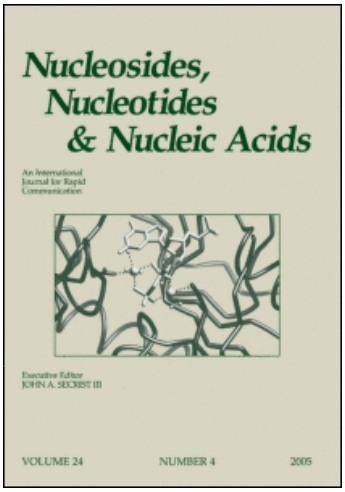

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Ribosylation of 3-Methylguanine and the Relative Stability of its 7- and 9-α-D-Ribofuranosides

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To cite this Article Boryski, Jerzy , Ostrowski, Tomasz and Golankiewicz, Bożenna(1989) 'Ribosylation of 3-Methylguanine and the Relative Stability of its 7- and 9-α-D-Ribofuranosides', Nucleosides, Nucleotides and Nucleic Acids, 8: 7, 1271 — 1280
To link to this Article: DOI: 10.1080/07328318908054331
URL: http://dx.doi.org/10.1080/07328318908054331

NUCLEOSIDES & NUCLEOTIDES, 8(7), 1271-1280 (1989)

RIBOSYLATION OF 3-METHYLGUANINE AND THE RELATIVE STABILITY OF ITS 7- AND 9-β-D-RIBOFURANOSIDES

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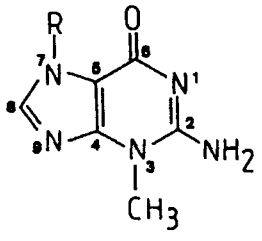
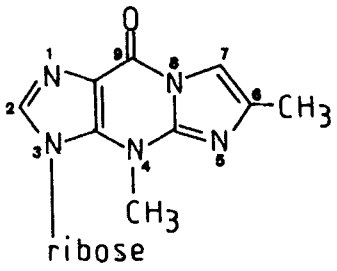
ABSTRACT: Ribosylation of 3-methylguanine 1a was investigated by enzymatic and chemical methods. Compound 1a did not act as a substrate for purine nucleoside phosphorylase. N-2-Protected 3-methylguanines 4 and 6 underwent exclusive N-7 glycosylation by fusion and chloromercury methods to give 5 and 7. Fully acetylated 7-β-D-ribofuranoside 5 was also obtained by thermal transglycosylation of the corresponding 9-β-D-ribofuranoside 9. The reverse isomerization 5 → 9 did not occur. The differences in the relative stability towards acidic hydrolysis between 7- and 9-(β-D-ribofuranosyl)-3-methylguanines are distinctly higher than those described so far for the other 7-9 isomeric nucleosides.

3-Substituted adenines and corresponding hypoxanthines have been early[1] found to undergo ribosylation exclusively at the 7 position. Such course of the reaction has been ascribed to a strong steric interference in 3,9-disubstituted purine derivatives in general[2]. There has been no report of the ribosylation of 3-methylguanine 1a.



1a R = H

1b R = ribose



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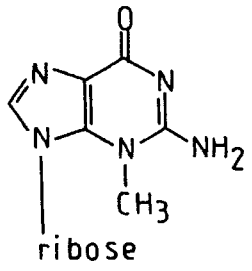

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It could have been expected that in routine glycosylations the directive influence of 3-methyl group will be similar to that observed for the other purines and will result in the formation of the hitherto unknown 7-(β-D-ribofuranosyl)-3-methylguanine 1b. In nature, however, 3-methylguanine occurs in form of 9-β-D-riboside, as a structural unit of the fluorescent, acid-labile Y-nucleosides, e.g. wyosine 2, characteristic for a number of transfer RNAs specific for phenylalanine[3]. The first synthesis of 9-(β-D-ribofuranosyl)-3-methylguanine (3-methylguanosine, 3) has been accomplished from 5-amino-4-cyano-1--(-β-D-ribofuranosyl) imidazole by a multistep route[4]. It has been shown very recently that direct N-3 methylation of guanosine may be enforced by reacting its 1,N-2-isopropeno derivative with organozinc reagent[5], followed by removal of the 1,N-2 blocking system[6].

It seemed of interest whether the anticipated 7-substitution in routine glycosylations would be the exclusive direction of the reaction and whether thermal 7⇆9 transglycosylation would occur. The latter reaction had been observed for some fully acylated purine nucleosides related to guanosine[7]. Different regioselectivity of the enzymatic ribosylation might have been also taken into account.

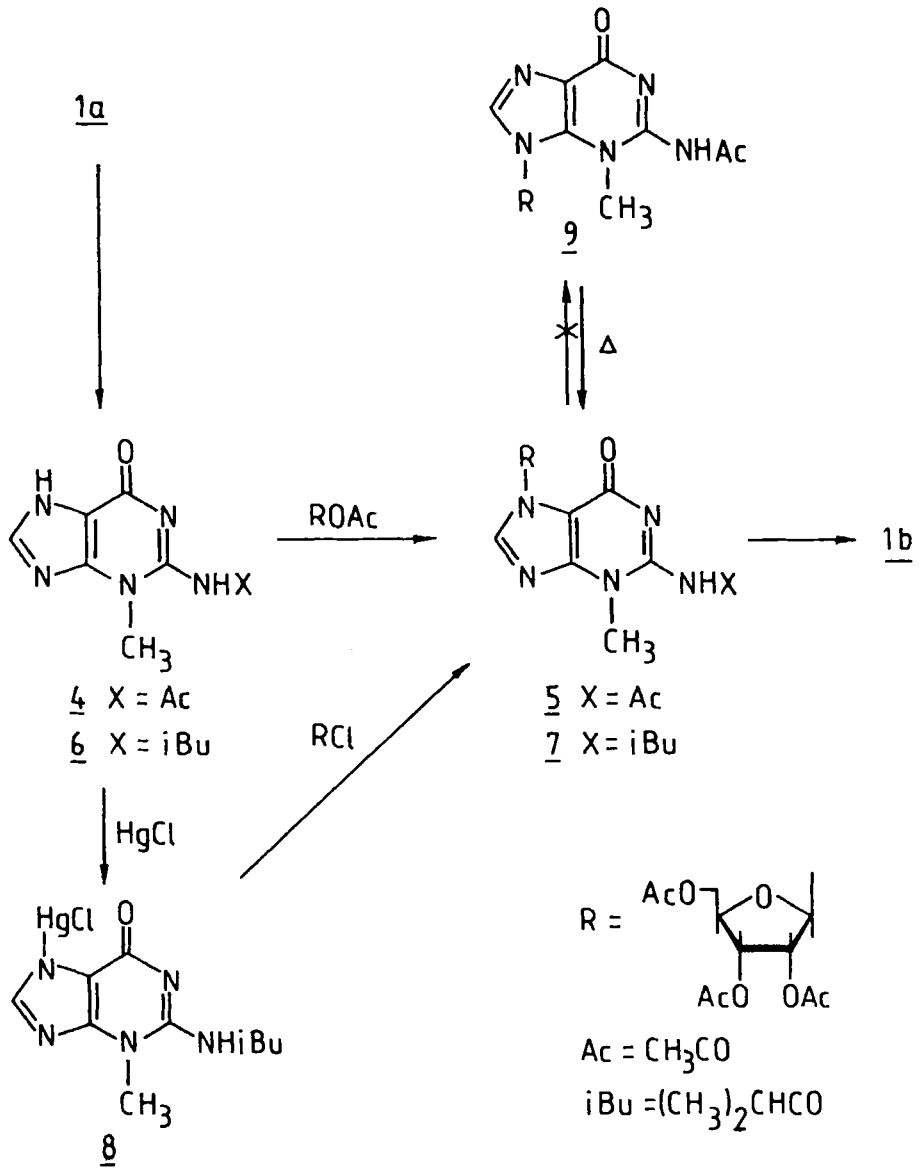
In exploring the latter possibility we subjected 3-methylguanine to purine nucleoside phosphorylase (PNP). It has been shown by Kalckar[8] that guanine plus ribose-1-phosphate is converted to guanosine in the presence of this enzyme according to the reversible reaction:

Guanine + ribose-1-phosphate ⇌ guanine riboside + inorganic orthophosphate.

Numerous modified purine ribosides and deoxyribonucleosides were synthesized in this way. We found however that 3-methylguanine did not act as a substrate for PNP. It remained unchanged after 48 hours under conditions which completely transformed a control sample of hypoxanthine into inosine within 2 hours.

The approaches we used to ribosylate 1a chemically are shown in Scheme I.

In the first variant of the fusion reaction 3-methylguanine 1a was transformed into N-2-acetyl derivative 4. Compound 4 was then fused with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in the presence of a catalytic amount of p-toluenesulfonic acid at 220° for 15 minutes. The products were separated on a silica gel short column to give peracetylated



SCHEME I

nucleoside 5 in 39% yield and small amounts of non-nucleosidic compounds. Deprotection of 5 by heating with 33% aqueous dimethylamine solution for 1 hour under reflux furnished 7-(β-D-ribofuranosyl)-3--methylguanine 1b in 63% yield. The UV spectrum of 1b λ_{max} (H_2O) 219 nm, 239, 273 was very close to that of 3-methylguanine[9] and different from that of 3-methylguanosine 3 λ_{max} (H_2O) 217 nm, 250, 265[4b]. ^1H NMR spectrum of 1b when compared with that of 3[4b] showed 0.17 ppm upfield shift of N-Me signal and approx 0.2 ppm downfield shifts of 1'-H and 8-H signals. The latter is a general characteristic criterion for differentiation of 7 and 9 ribosides and their analogues[10]. The ribosylation must have taken place exclusively at the N-7, because ^1H NMR did not show even traces of 3-methylguanosine.

When N-2-isobutyryl-3-methyguanine 6 was used instead of N-2-acetyl derivative 4, the fusion reaction could be performed at lower temperature but resulted in a complex mixture. In addition to 7-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-N-2-isobutyryl-3-methylguanine 7 (32%), a transacylation product 5 (19%) was isolated. Also in this experiment no traces of the N-9 substitution could be found. Deprotection of both 7 and 5 with aqueous methanolic ammonia (room temperature, 24 hours) resulted in compound 1b in all respects identical with that obtained by the first method. In the third effort, chloromercury approach, N-2-isobutyryl-3-methylguanine 6 was transformed into corresponding chloromercury salt 8 in 55% yield. It was subsequently condensed with 1-β-chloro-2,3,5-tri-O-acetyl-D-ribose in refluxing xylene. The fully protected 7-β-D-riboside 7 thus obtained (49% yield after chromatographic purification) was in all respects identical with the compound furnished by the fusion procedure.

Transglycosylation experiment attempted on 7-(2',3',5'-tri-O--acetyl-β-D-ribofuranosyl)-N-2-acetyl-3-methylguanine 5 failed. Compound 5 remained unchanged when heated at 200°C for 20 minutes. Higher temperatures resulted in partial decomposition without any traces of 9-riboside.

On the contrary, 9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-N-2--acetyl-3-methylguanine 9 when heated at 230° for 3 min. was completely transformed into its 7-isomer 5, in all respects identical with the compound obtained by ribosylation described above.

The results presented above have provided an additional example for the general regularity that 3-substitution in purine directs the next substituent exclusively towards the 7-position under a variety of conditions.

The stability of the glycosidic bond of 7-(β-D-ribofuranosyl)-3--methylguanine 1b towards acidic hydrolysis was found to be slightly higher than that of the unsubstituted 7-(β-D-ribofuranosyl)guanine, $t_{1/2}$ of the glycosidic bond cleavage was respectively 31 and 23.9 h[11] in 1N HCl aq at 45°C. 3-Methylguanosine 3 has been reported to undergo acidic hydrolytic cleavage with $t_{1/2}$ 42s in 0.1N HCl aq at 25°C[4b]. The above data emphasize the extreme differences of the glycosidic bond cleavage between compounds 1b and 3. They provide an interesting supplement to the former study on the relative stability of certain 7- and 9-(β-D-ribofuranosyl) purines for which the highest differences in the rate of the acidic hydrolyses were less than two orders of magnitude[11].

Glycosidic bond of 1b undergoes unusual labilization on acetylation. Compound 5 was completely depurinated after 72 h in 0.1N HCl aq at 25°C[12]. The effect is opposite to that known for 9-ribo- and 9-deoxyribonucleosides in which protection of the hydroxyl functions of the sugar moiety with electronegative groups results in stabilization of the glycosidic linkages[13,14].

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Jeol FX-90Q Spectrometer in dimethyl-d_6 sulfoxide. The chemical shift values are expressed in δ, parts per million, relative to tetramethylsilane as an internal standard. Ultraviolet (UV) spectra were recorded in water on a Zeiss Specord UV-Vis Spectrophotometer. The rate of the glycosidic bond hydrolysis was measured on a Zeiss VSU-2P spectrophotometer using Vierordt method. Thin-layer chromatography (TLC) was conducted on Merck precoated silica gel F_{254} plates (0.25 mm) using the following solvent systems measured by volume: A, chloroform-methanol (9:1); B, n-butanol-glacial acetic acid-water (5:3:2). For a preparative short column chromatography Merck TLC Silica gel type 60H was used.

Evaporations were performed under reduced pressure below 40°C with a rotary evaporator. Elemental analyses were performed by the Microanalytical Laboratories of the Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw and of A. Mickiewicz University, Poznań. 3-Methylguanine was prepared according to [9b], 1-chloro-2,3,5--tri-O-acetyl-β-D-ribofuranose according to [15].

Attempted glycosylation of 3-methylguanine (1a) with α-D-ribofuranose-1-phosphate employing purine nucleoside phosphorylase. Compound 1a (1.65 mg, 10 μmol) was dissolve in Sörensen phosphate buffer (3 mL, 0.07M, pH 7.5), purine nucleoside phosphorylase from bovine spleen (1 unit) was added followed by α-D-ribofuranose-1--phosphate di(monocyclohexylammonium)salt (3.3 mg, 15 μm). The reaction mixture was stirred at 25°C for 48 h. No traces of 3-methylguanine riboside could be observed by TLC (dichloromethane/ethanol anh. 4:1). In the control experiment, using hypoxanthine as a substrate, glycosylation was complete after 2 h.

N-2-Acetyl-3-methylguanine (4). 3-Methylguanine 1a (660 mg, 4.0 mmol) was heated with acetic anhydride (12 mL) under reflux for 10 h. The solvent was removed in vacuo, the residue was dissolved in EtOH and suspended on silica gel by evaporation. It was then applied onto a short silica gel column and eluted with chloroform-methanol (95:5) followed by (9:1). The fractions containing the homogenous product were pooled and evaporated. The residue was recrystallized from ethanol to give 4 (310 mg, 38%): mp 284°C dec without melting; TLC R_f 0.56(A), 0.64(B); UV λ_{max} (H_2O) 226 nm (ε 13 700), 270 (11 500); ^1H NMR (Me_2SO-d_6) δ 2.12 (s,3, $NHCOCH_3$) 3.62 (s,3,N-3 CH_3), 8.21 (s,1,H-8), 10.67 (brs,1, NH), 13.56 (brs,1,NH). Anal. Calcd for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.80. Found: C, 46.24; H, 4.35; N, 33.61.

N-2-Isobutyryl-3-methylguanine (6). A suspension of 1a (200 mg, 1.21 mmol) in dry pyridine (10 mL) was treated with isobutyryl chloride (516 mg, 4.84 mmol), then stirred at room temperature for 1 h. The resulting clear solution was evaporated to an oil, which was then coevaporated with chloroform-toluene (4:1, 2x10 mL). The residue obtained after evaporation was dissolved in chloroform-methanol (95:5)

and chromatographed on a silica gel column (2.5x6 cm) in this solvent system. Fractions containing chromatographically homogenous material were evaporated to a white solid, which was crystallized from ethyl acetate to yield 214 mg (75%) of 7: mp 212°C; TLC R_f 0.63(A), 0.77(B); UV λ_{max} (H_2O) 225 nm (ε 16 300), 268 (13 300); ^1H NMR (Me_2SO-d_6) δ 1.13 (d,6,CH($\underline{CH_3}$)$_2$), 3.36 (m,1,$\underline{CH}$(CH_3)$_2$), 3.64 (s,3, N-3 CH_3), 8.20 (s,1,H-8), 13.55 (brs,2,2xNH). Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.77. Found: C, 50.94; H, 5.50; N, 29.46.

7-(β-D-Ribofuranosyl)-3-methylguanine (1b). Method A. 7-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-N-2-acetyl-3-methylguanine (5). A mixture of 4 (350 mg, 1.7 mmol), 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (805 mg, 2.5 mmol) and p-toluenesulfonic acid monohydrate (26 mg, 0.14 mmol) was heated at 220°C for 15 min. According to TLC the reaction mixture contained the main product and traces of compounds with lower R_f. The melt was dissolved in chloroform and chromatographed on a silica gel short column using chloroform-methanol 98:2 as eluent. The appropriate fractions were combined and evaporated to furnish chromatographically homogenous 5 as a solid foam (305 mg, 39%): mp 128-130°C; TLC R_f 0.86(A), 0.72(B); UV λ_{max} (H_2O), 230 nm (ε 12 700), 272 (9 000); ^1H NMR (Me_2SO-d_6) δ 2.04, 2.08, 2.10, 2.13 (4xs, 12, 4x$COCH_3$), 3.61 (s,3,N-3 CH_3), 4.34 (m,3,H-4', 5'), 5.50 (t,1,H-3'), 5.79 (t,1,H-2'), 6.33 (d,1,H-1'), 8.56 (s,1,H-8), 13.62 (brs,1NH). No traces of signals indicative for N-9 ribosylation. Compound 5 (120 mg, 0.26 mmol) was heated under reflux with dimethylamine (33% aq solution) for 1 h. Dimethylamine was removed in vacuo, and the residue was treated with cool 60% aq ethanol. The completely unblocked product 1b did not go into the solution. It was filtered off and dried (48 mg, 63%): mp >300°C; TLC R_f 0.0(A), 0.44(B); UV λ_{max} (H_2O) 219 nm (ε 18 800), 239 (9 000), 273 (9 400); ^1H NMR (Me_2SO-d_6) δ 3.38 (br, H_2O), 3.53 (s,3,N-3 CH_3), 3.60 (m,2,H-5'), 4.01 (m,1,H-4'), 4.11 (m,1,H-3'), 4.34 (m,1, H-2'), 5.08 (m,2,OH 3',5'), 5.51 (d,1,OH 2'), 6.11 (d,1, H-1'), 6.97 (s,2,NH_2), 8.25 (s,1,H-8). Anal. Calcd for $C_{11}H_{15}N_5O_5 \cdot 0.5\ H_2O$: C, 43.13; H, 5.26; N, 22.86. Found: C, 43.45; H, 5.26; N, 22.66.

Method B. A mixture of 6 (15 mg, 64 μmol), 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (238 mg, 64 μmol) and p-toluenesulfonic acid monohydrate (1 mg, 5 μmol) was heated under diminished pressure (12 mm

Hg) at 190° for 45 min. The resulting oil was dissolved in chloroform-methanol (98:2) and chromatographed on a silica gel column (0.8x8 cm) in this solvent system; 3 ml fractions were collected. Fractions 4 and 5 contained the main product, 7-(2',3',5'-tri-O-acetyl-β-D--ribofuranosyl)-N-2-isobutyryl-3-methylguanine 7, as a chromatographically homogenous oil (10.1 mg, 32%): TLC R_f 0.92(A); UV λ_{max} (H_2O) 226 nm, 269. Fraction 7 contained a side product, R_f 0.86(A) identical with tetraacetyl derivative 5 obtained in method A (5.6 mg, 19%). Compound 7 (10 mg) was dissolved in methanol (1 mL) and treated with concd aq ammonia (10 mL). After 24 h at room temp. the deprotection was complete to give 1b identical (UV,TLC) with that obtained in method A. Compound 5 was deblocked into 1b in the same manner.

Method C. Compound 6 (77.7 mg, 0.33 mmol) was dissolved in hot methanol (7 mL) and treated with 2N NaOH (165 μl) and with a solution of mercuric chloride (89.7 mg, 0.330 mmol) in methanol (1 mL). The solution was concentrated to a volume of ca 1 mL and diluted with water (10 mL). A white precipitate that appeared was filtered off, washed carefully with water and dried to give 85.5 mg (55%) of the respective chloromercuric salt. Content of mercury (determined as mercuric sulfide): Calcd. 42.65%. Found 41.93%.

To an anhydrous suspension of the chloromercuric salt 8 (45.5 mg, 0.097 mmol) in xylene (2 mL) was added 1-chloro-2,3,5-tri-O-acetyl-D--ribofuranose (34.2 mg, 0.116 mmol), in xylene (0.5 mL). The suspension was refluxed for 1 h, then cooled and evaporated to dryness. The main product was purified by short column chromatography (0.8x8 cm) in chloroform-methanol (98:2) to give 23.4 mg (49%) of the fully protected 7-riboside 7 λ_{max} (H_2O) 226, 269 nm. A sample of the product (10 mg) was deprotected as in method B to give 7-(β-D-ribofuranosyl)-3-methylguanine 1b, identical with that obtained in the methods A and B (UV, TLC).

Attempted 7→9 transglycosylation of compound 5. Samples of 5 (10 mg, 21.5 μmol) were heated for 20 min on an oil bath at 200, 230 and 250°C. The resulting oil was dissolved in chloroform and analysed by TLC (solvent A). At 200°C the substrate remained unchanged, at higher temperatures a spot of N-2-acetyl-3-methylguanine 4 appeared.

9→7 Transglycosylation of 9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-N-2-acetyl-3-methylguanine (9) to 5. A crystalline sample of 9 (10 mg, 21.5 μmol; obtained by treatment of 3[6] with acetic anhydride in pyridine and crystallized from ethanol, mp 162°C, lit.[4a] 153-4°C) was heated at 230°C for 3 min. The resulting oil was dissolved in chloroform and analyzed by TLC in solvent A, what revealed a complete disappearance of the starting material (R_f 0.67), a major spot corresponding to the 7-riboside 5 (R_f 0.86) and traces of N-2-acetyl-3-methylguanine 4 (R_f 0.56). The ^1H NMR spectrum of the reaction mixture in $CDCl_3$ was identical with that of compound 5.

Glycosidic bond cleavage of 5. A solution of 5 (1 mg, 2.14 μmol) in 0.1N aq HCl (0.3 mL) was maintained at 25.0°C. A progress of the reaction was monitored by TLC in solvents A and B. Samples of the reaction mixture were taken at intervals of time and neutralized by mixing them with 0.2 mL of phosphate buffer of pH 7. These solutions were spotted on TLC plates along with solutions of 1a and 1b as standards. The spot of 5 disappeared after 3 h and was replaced by several spots of lower R_f values. Their relative intensity was changing with the reaction progress. After 72 h the only intensive spot was that of the same R_f value as the 3-methylguanine 1a control.

Acknowledgements

This work was supported by the Polish Academy of Sciences, Project CPBR 3.13.4.2.5. The enzymatic experiments were performed by one of us (B.G.) at the University of Osnabrück (West Germany). We are greatly indebted to Professor F. Seela and Dr. H. Rosemeyer for valuable discussions.

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Received April 18, 1988.