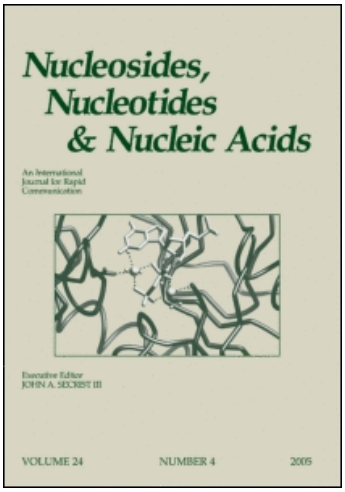

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TAUTOMERISM AND REGIOSELECTIVITY IN RIBOSYLATION OF GUANINE

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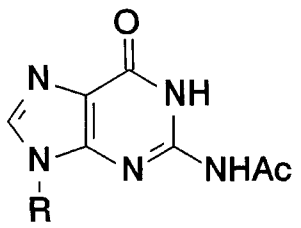
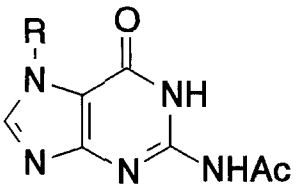
ABSTRACT: N^2-acetyl- and 9,N^2-diacetylguanines were subjected to reaction with tetra-acetylribose in the presence of p-toluenesulfonic acid. Unlike the ribosylation of diacetylguanine, which gives 7-riboside as a kinetic product, the reaction of monoacetyl-guanine produces directly a mixture of 7- and 9-ribosides. This reflects $N^7H \rightleftarrows N^9H$ tautomerism of the guanine substrate and supports the hypothesis that only the unsubstituted nitrogens of the imidazolium portion of guanine (either N7 or N9) react directly with a sugar cation.

It has been shown that glycosyl exchange reactions in the series of guanine nucleosides proceed regioselectively.[1] Thus tetraacetylguanosine gives 7-substituted acyclonucleoside as a kinetic product in the reaction with 2-acetoxyethyl acetoxymethyl ether, while tetraacetyl derivative of 7-(β-D-ribofuranosyl)guanine is directly transformed to the 9-substituted product. These findings lead to hypothesis that synthesis of guanine nucleosides proceeds *via* initial glycosylation of the unsubstituted nitrogen of imidazole ring (either N7 or N9), no matter what the starting substituent covalently bound to the other nitrogen is. It has been already confirmed for guanine derivatives substituted at N7 or N9 with triacetylribofuranosyl and (2-acetoxyethoxy)methyl group.[1] Presumably, this rule of regioselectivity may also be applicable to the other starting substituent commonly met in nucleoside synthesis, e.g. silyl or acyl groups, mercury or silver atoms, and even proton.

In the present work regioselectivity of ribosylation was studied in the case of acetylated derivatives of guanine. Reaction of N^2-acetylguanine (**1**) or 9,N^2-diacetylguanine (**2**) with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (10 eqs.) was performed in refluxing chlorobenzene in the presence of p-toluenesulfonic acid (0.1 eq.) (SCHEME 1). The yield of 7- and 9-ribosylated regioisomers (compounds **3b** and **3a**, respectively) was determined by the HPLC method, using a Waters HPLC set with Nova Pak C_{18} column. Chromatographic separations

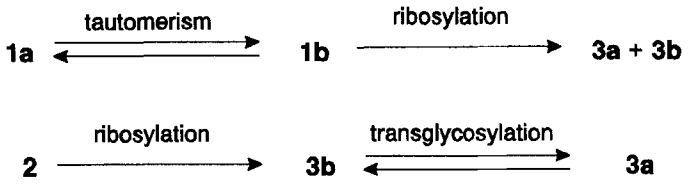


1a R = H
2 R = CH_3CO
3a R = 2',3',5'-tri-O-acetyl-
-β-D-ribofuranosyl



1b R = H

3b R = 2',3',5'-tri-O-acetyl-
-β-D-ribofuranosyl



SCHEME 1

were performed in a methanol - water reverse gradient at a flow rate 1 mL/min and UV absorption was measured at 260 nm.

When left for 12 h, both reactions resulted in a similar distribution of products: tetraacetylguanosine (**3a**) and its 7-isomer (**3b**) in the ratio 3:2, approximately. That might suggest that there was no regioselectivity of ribosylation and both reactions proceeded according to the same mechanism. However, the HPLC analysis after a very short reaction time indicated some basic differences in ribosylation of monoacetyl and diacetyl substrates. In the initial period of reaction, the ratio of 7/9 substitution was much higher for the diacetyl substrate (**2**) than in the case of monoacetylguanine. The 7-riboside (**3b**) was found as a single product after 1 min of the reaction when ribosylation started from **2** (FIG. 1A).

This indicates that the 7-substituted compound is a kinetic ribosylation product of the 9-substituted substrate, what is in line with the discussed rule. The final distribution of regioisomers can be rationalized as a result of reversible 7 ⇄ 9 transglycosylation,[1-7] which proceeds *via* 7,9-diribofuranosylpurine intermediates.[1,4]

In contrast, ribosylation of monoacetylguanine led directly to a mixture of 7- and 9-ribo products. After 1 min of reaction HPLC showed the presence of **3b** and **3a** in the ratio 5:1.

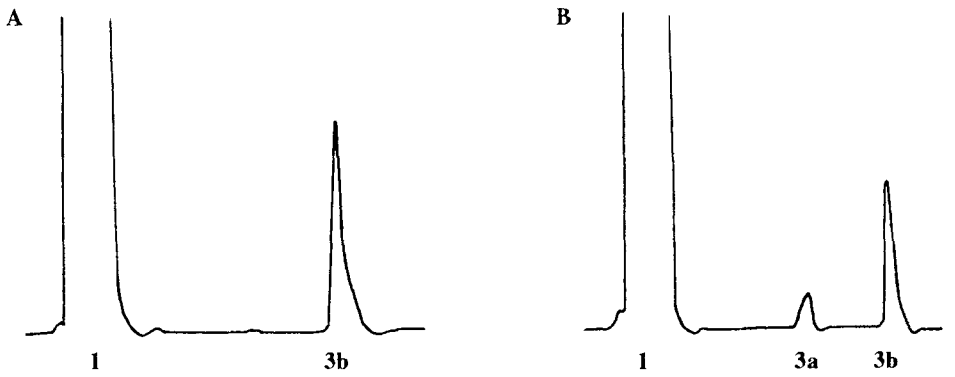


FIG. 1. HPLC analysis of the reaction mixtures after 1 min of ribosylation: A, 9,N^2-diacetylguanine (**2**); B, N^2-acetylguanine (**1**). In the reaction A unreacted **2** was analysed as the monoacetyl derivative (**1**) due to rapid hydrolysis of its 9-acetyl group in aq. MeOH.

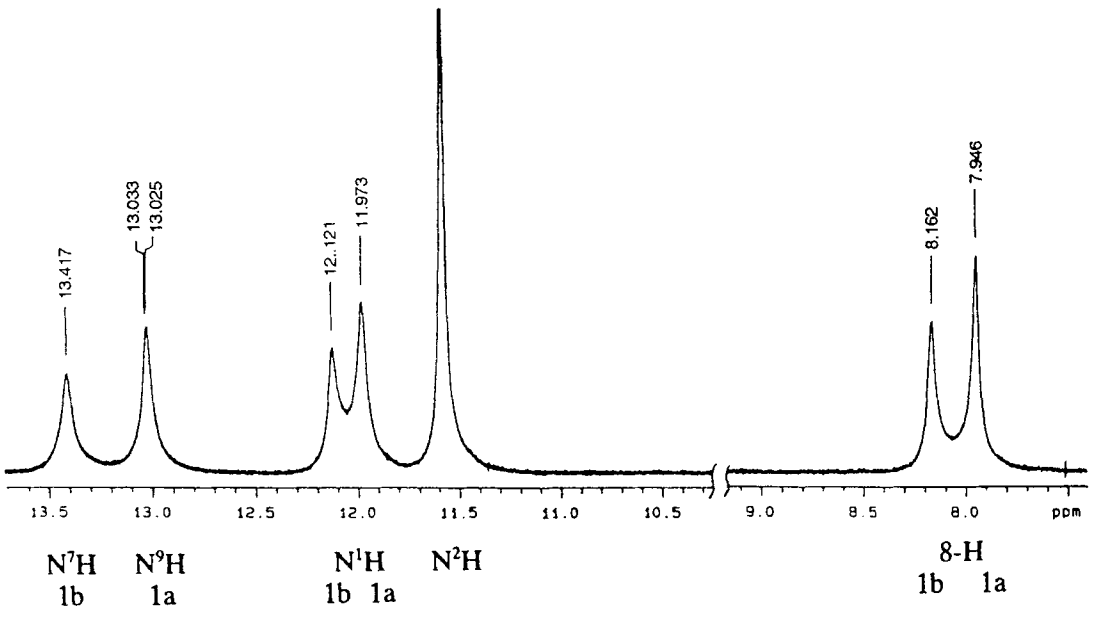


FIG. 2. ^{1}H NMR spectrum (300 MHz, δ, ppm, DMSO-d_6) of N^2-acetylguanine.

The observed simultaneous formation of 7- and 9-substituted products in the latter case can be explained as an effect of the $N^7H \rightleftarrows N^9H$ tautomerism of the substrate. Indeed, the ^1H NMR spectrum of N^2-acetylguanine measured in DMSO-d_6 at 20°C revealed the presence of N^9H (**1a**) and N^7H (**1b**) tautomers in the ratio 56:44, respectively. The tautomerism may also occur under the ribosylation conditions (but its equilibrium seems to be shifted towards the N^9H form) and each tautomer reacts individually with the sugar cation. Thus the N^7H tautomer gives directly the 9-ribosylated product, and *vice versa*, ribosylation of the N^9H tautomer leads to the 7-substituted regioisomer. In this way the proposed rule of regioselectivity is still observed. The $7 \rightleftarrows 9$ transglycosylation is again responsible for the final distribution of isomers.

These experimental data support the mechanism of ribosylation of 6-oxopurines presented by Dudycz and Wright[4] and underline the fact that only N7 and N9 atoms participate in glycosylation and glycosyl exchange reactions.[1] The formation of 3-ribosylated kinetic products[8] seems to be very doubtful in the series of 6-oxopurines. The results obtained recently[1] as well as in this work lead to the conclusion that the structure of the kinetic product in ribosylation of 6-oxopurines depends on the site of substitution of the substrate. Only the unsubstituted (i.e. of hybridization sp^2) nitrogen atoms (either N7 or N9) may be a nucleophilic center which reacts with a sugar cation.

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