Regioselectivity and mechanism of transpurination reactions in the guanine nucleosides series

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The transpurination reaction of the fully acetylated derivatives of guanosine and its 7-β-D-ribofuranosyl regioisomer with 2-acetoxyethyl acetoxymethyl ether has been studied using high performance liquid chromatography (HPLC). The regioselectivity of glycosyl exchange observed in the early stages of the reaction suggests that the unsubstituted nitrogen atoms of the imidazole portion (N7 and N9) are, exclusively, sites of direct glycosylation in the case of guanine derivatives. The results lead to the conclusion that the mechanism of the glycosylation reaction of guanine is different to that of adenine.

Transpurination is a glycosyl exchange reaction, in which the purine moiety of the nucleoside is transferred from one sugar to another. This process was originally noticed by Lichtenhaller and Kitahara, who described the migration of hypoxanthine from ribose to glucose when 2′,3′-*O*-isopropylideneinosine was reacting with 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose in the presence of mercury(II) cyanide.[1] More recently, the transpurination reaction was applied as a convenient method for the synthesis of acyclonucleosides of pronounced antiviral activity, *e.g.* acyclovir and ganciclovir.[2–4]

Transpurination, and its better known counter reaction, transglycosylation,[2,3,5–13] are examples of glycosyl migration reactions and play an important role in nucleoside chemistry. For example, transglycosylation is the second step of *N*-glycosyl linkage formation, in which the kinetic glycosylation product, obtained directly from an appropriate heterocyclic base and a protected sugar derivative, undergoes a rearrangement to the thermodynamically more stable nucleoside. This isomerisation process appears to be an intermolecular reaction catalysed by Lewis acids.[12,13]

In the purine series, the mechanism of glycosyl exchange seems to be well documented in the case of adenine and its derivatives. According to the proposed mechanism, adenine undergoes initial glycosylation at position 3. The unstable 3-glycosyladenine derivative then reacts with another sugar cation to form a 3,9-diglycosylpurine quaternary salt intermediate. Its decomposition gives the final 9-substituted product. The 3→9 sequence of glycosylation and transglycosylation has also been suggested for purine bases other than adenine.[12,13]

However, as shown by Dudycz and Wright in a more recent study,[14] the respective 7-ribonucleosides (not 3-ribo derivatives) were the kinetic products in ribosylation of persilylated 2-bromohypoxanthine, N^2-acetylguanine and N^2-(*p*-butylphenylguanine). In addition, they isolated the corresponding 7,9-diribofuranosylpurines as unstable reaction intermediates. Those findings may suggest that the transglycosylation of 6-oxopurines, *i.e.* guanine, hypoxanthine and their derivatives, proceeds according to a different mechanism to that of adenine.

In order to elucidate the mechanism of glycosyl exchange in the case of guanine, the previously reported[2,3] conversion of guanosine into acyclovir has been investigated as a model reaction.[15] In this approach, two different substituents, *i.e.* 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl and (2-acetoxyethoxy)methyl, were present in the reaction medium. That pair of substituents has been chosen for three reasons. First, both groups are of similar reactivity in glycosyl exchange reactions.[11,19] Secondly, application of these groups allows us to avoid anomerization: the (2-acetoxyethoxy)methyl substituent does not introduce a new centre of asymmetry, whereas tri-*O*-acetyl-β-D-ribofuranosyl derivatives react in line with the Baker's rule,[16] producing almost exclusively β-ribosides. Thirdly, the ribo- and acyclonucleosides can be readily distinguished by chromatographic means.

Results and discussion

In the transpurination experiments tetraacetylguanosine **1** or its 7-β-regioisomer **6** (obtained from **1** by using the thermal 7⇌9 isomerisation method)[3,11] were subjected to the action of a 10-fold molar excess of 2-acetoxyethyl acetoxymethyl ether.[17] The reactions were performed in dry chlorobenzene at 120 °C for 2 h, in the presence of toluene-*p*-sulfonic acid (10 mol%) as a catalyst. The reaction mixtures were analysed by HPLC (see Experimental).

Interestingly, after a prolonged reaction time (2 h) both experiments gave an almost identical distribution of products: the regioisomeric mixture of acyclonucleosides **3** and **8** as major products (in a ratio of *ca.* 1:1), as well as smaller amounts of ribonucleosides **1** and **6**. The pathways to reach that dynamic equilibrium state, however, were different in both cases, as judged by HPLC (Figs. 1 and 2).

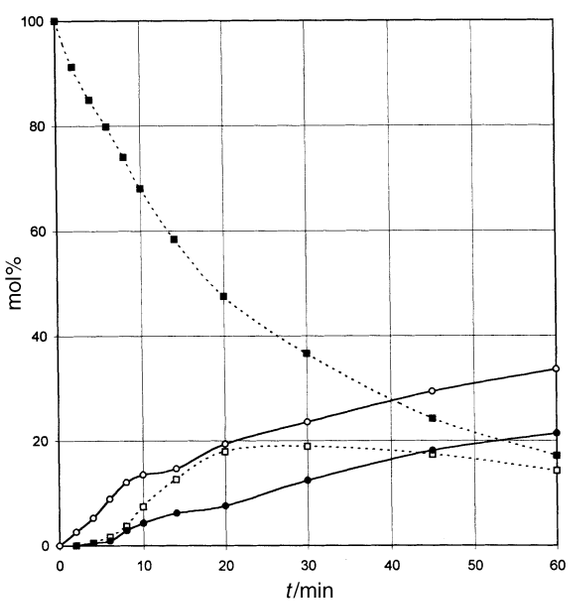


Fig. 1 Time-dependent product distribution for the reaction of 9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-N^2-acetylguanine (compound **1**) with 2-acetoxyethyl acetoxymethyl ether (see Experimental): **1** ■; **6** □; **8** ●; **3** ○. The mole fractions are based on HPLC data.

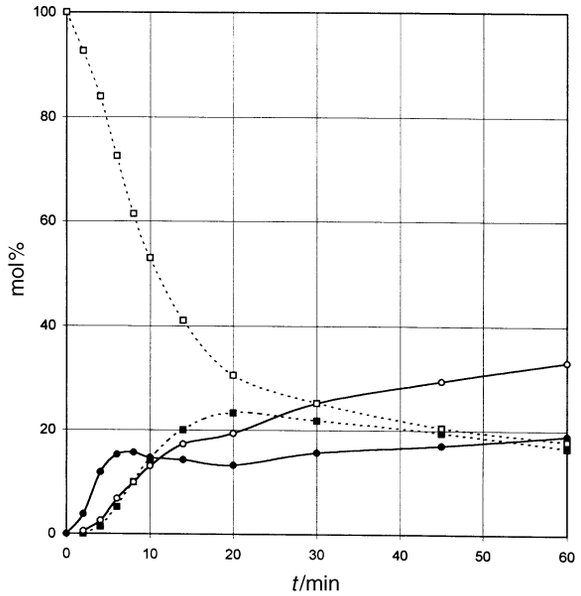
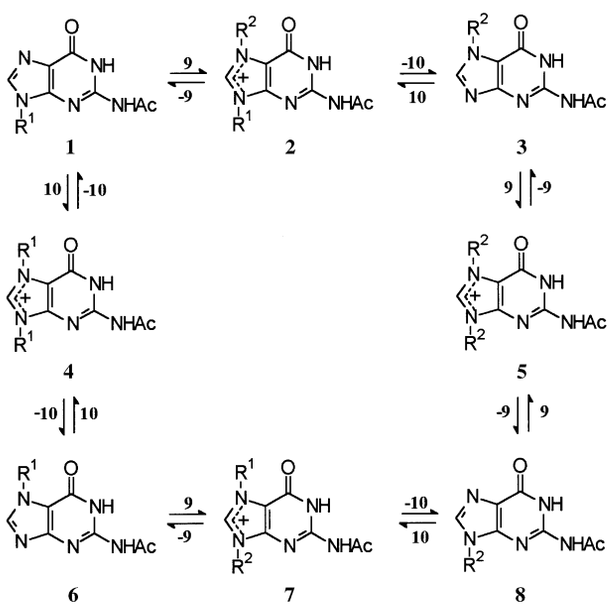


Fig. 2 Time-dependent product distribution for the reaction of 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-N^2-acetylguanine (**6**) with 2-acetoxyethyl acetoxymethyl ether: **1** ■; **6** □; **8** ●; **3** ○

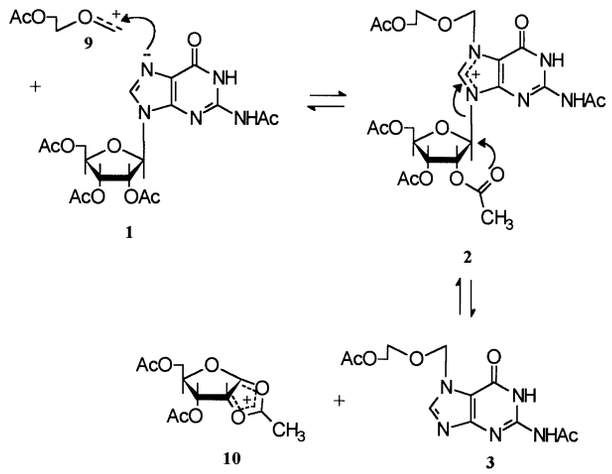
When the peracetylated 9-riboside **1** was used as the substrate, the 7-acyclonucleoside **3** was found as a single reaction product after 2 min (Fig. 1), whereas in the transpurination reaction of the 7-riboside **6** the 9-substituted acyclonucleoside **8** (diacetylacyclovir) was apparently the kinetic reaction product (Fig. 2). The results of the HPLC analysis were also confirmed by thin-layer chromatography (TLC) and ^{1}H NMR spectroscopy. Any putative compound with the structure of a 3-substituted N^2-acetylguanine was not detected in transpurination experiments.

The mechanism and sequence of these glycosyl exchange reactions may be depicted as shown in Schemes 1 and 2.



Scheme 1

Reaction of 2-acetoxyethyl acetoxymethyl ether with toluene-*p*-sulfonic acid generates carboxonium cation **9**, which is then attacked by the *unsubstituted nitrogen atom* of the imidazole ring, *i.e.* N7 in the case of the 9-riboside **1** (Scheme 2), and N9



Scheme 2

for the 7-riboside **6**. The resulting unstable 7,9-diglycosylguanine intermediates then undergo decomposition: the intermediate **2** decomposes to 7-acyclonucleoside **3** (Scheme 2), whereas compound **7** gives 9-acyclonucleoside **8**. Additionally, both exchange reactions produce the acyloxonium cation **10** (the structure of the respective carboxonium ion can be taken into consideration as well),[13] and therefore the reactions are reversible. These transpurination reactions are shown in Scheme 1 as horizontal arrows.

However, the liberated ribosugar cations **10** may in turn react with the starting nucleoside, which leads to the 'intramolecular'[18] 7⇌9 isomerization. This also applies to 7⇌9 isomerization of acyclonucleosides **3** and **8** in the presence of acyclosugar cations **9**. The vertical arrows in Scheme 1 denote the reversible 7⇌9 transglycosylation reactions.

As a result, the reaction mixtures in both cases contain all possible 9- and 7-regioisomers: two pairs of guanine ribo- and acyclo-nucleosides (compounds **1**, **6** and **8**, **3**, respectively). Under the conditions described, equilibrium is achieved after *ca*. 2 h. The acyclonucleosides **3** and **8** are predominant products because an excess of 2-acetoxyethyl acetoxymethyl ether has been applied (Figs. 1 and 2).

It can also be deduced from Scheme 1 that any direct 'diagonal' conversion is not possible, *e.g.* diacetylacyclovir **8** is produced from tetraacetylguanosine **1** either *via* 7-ribonucleoside **6**, or *via* 7-acyclonucleoside **3**. In both cases the conversion requires not just one, but two reactions: transpurination and 7⇌9 transglycosylation. The respective 7,9-disubstituted derivatives (**2**, **4**, **5**, **7**), not isolated in this study, are postulated as reaction intermediates for each single process of glycosyl exchange.

Furthermore, a detailed comparison of Figs. 1 and 2 suggests that 7-riboside **6** reacts more readily with the acyclosugar cation **9** than its 9-regioisomer **1**. It has been previously shown for the irreversible 2→1 transglycosylation of riboindazoles[19] that kinetic products of glycosylation undergo a fast reaction with other sugar cations, whereas thermodynamic products do not. Therefore, in the case of guanine nucleosides the 9-regioisomers may be considered as the 'more thermodynamic' products than the 7-substituted compounds. However, that difference is not significant and all glycosyl exchange reactions in the guanine series are reversible.

The results obtained in this study lead to the following conclusions.

(*i*) In the guanine series only the nitrogen atoms of the imidazole portion (*i.e.* N7 and N9) participate in glycosyl exchange reactions. This perhaps applies also to other 6-oxopurine nucleosides.

(*ii*) There is no experimental evidence that N3 of guanine takes part in glycosylation or transglycosylation reactions.

Table 1 ^{1}H NMR chemical shifts [δ (ppm)][a]

Compound	N^1H	N^2H	8-H	1′H[b]	2′H	3′H	4′H	5′aH[b]	5′bH[b]	*N*-Acetyl	*O*-Acetyl
1	12.11	11.62	8.28	6.11	5.84	5.50	4.39		4.33	2.21	2.14, 2.07, 2.05
	s, 1	s, 1	s, 1	d, 1; 6.6[c]	t, 1	dd, 1	m, 2		dd, 1	s, 3	3 × s, 9
6	12.25	11.69	8.53	6.31	5.81	5.47	4.33	4.43	4.25	2.18	2.11, 2.04, 2.03
	s, 1	s, 1	s, 1	d, 1; 5.7[c]	t, 1	dd, 1	q, 1	dd, 1	dd, 1	s, 3	3 × s, 9
8	12.07	11.79	8.14	5.48	—	—	4.07	3, 70		2.18	1.95
	s, 1	s, 1	s, 1	s, 2			m, 2	m, 2		s, 3	s, 3
3	12.17	11.63	8.38	5.69	—	—	4.06	3.72		2.17	1.95
	s, 1	s, 1	s, 1	s, 2			m, 2	m, 2		s, 3	s, 3

[a] Recorded at 300 MHz in $(CD_3)_2SO$; ref. $SiMe_4$. Figures following the observed multiplicities are numbers of protons as estimated by integration. [b] Or the respective methylene groups for acyclonucleosides. [c] Coupling constant $J_{1'2'}$ in Hz.

Table 2 ^{13}C NMR chemical shifts [δ (ppm)][a]

Compound	$NCOCH_3$	$OCOCH_3$	C-6	C-4	C-2	C-8	C-5	C-1′[b]	C-4′[b]	C-2′	C-3′	C-5′[b]	$NCOCH_3$	$OCOCH_3$
1	173.50	170.03	154.68	148.58	148.22	137.79	120.38	84.57	79.83	72.16	70.30	63.05	23.82	20.47
		169.39												20.33
		169.19												20.10
6	173.39	169.98	158.37	152.17	147.51	144.12	110.56	87.54	79.46	73.06	69.62	62.94	23.66	20.43
		169.30												20.26
		169.10												20.08
8	173.44	170.12	154.77	148.76	148.03	139.96	120.08	73.32	66.58	—	—	62.57	23.69	20.44
3	173.30	170.11	157.35	152.40	147.16	145.02	111.05	74.84	66.29	—	—	62.63	23.62	20.45

[a] Recorded at 75.43 MHz, ref. $SiMe_4$. [b] Or the respective carbon atoms of the acyclic chain.

Table 3 UV spectral data, TLC chromatography, and elemental analyses

Compound	λ_{max}(MeOH)/nm (ε/dm^3 mol^{-1} cm^{-1})	R_f-value in system[a] A	B	Formula	Found %C	%H	%N	Requires %C	%H	%N
1	259 (16 100), 279 (11 400)	59	37	$C_{18}H_{21}N_5O_9$	48.1	4.7	15.3	47.9	4.7	15.5
6	263 (13 400), 280 (sh,[b] 10 300)	65	53	$C_{18}H_{21}N_5O_9$	48.0	4.8	15.3	47.9	4.7	15.5
8	259 (16 200), 278 (11 600)	35	23	$C_{12}H_{15}N_5O_5$	46.7	4.9	22.2	46.6	4.9	22.6
3	263 (13 500), 280 (sh,[b] 10 100)	53	42	$C_{12}H_{15}N_5O_5$	46.7	4.8	22.4	46.6	4.9	22.6

[a] See Experimental section. [b] Shoulder.

(*iii*) The unsubstituted nitrogen (*i.e.* of hybridization sp^2) is the nucleophilic centre that reacts directly with sugar cations. Therefore, the structure of kinetic glycosylation products in the guanine series depends on the substitution of substrates: the 9-substituted derivatives of guanine give 7-glycosylated kinetic products, and *vice versa*, the 7-substituted substrates undergo direct glycosylation at N9.

(*iv*) The formation of 7,9-diglycosyl-6-oxopurine quaternary salts, postulated as the glycosylation intermediates by Dudycz and Wright[14], allows us to rationalise the regioselectivity observed in this study.

(*v*) The sequence and mechanism of glycosylation reaction in the guanine series is different to that documented for adenine.[12,13] While adenine is initially glycosylated at N3 and the resulting kinetic product undergoes irreversible rearrangement to the 9-substituted adenine, guanine is directly glycosylated at either N7 or N9 (see point *ii*), and the reversible 7⇌9 transglycosylation accounts for the final distribution of regioisomers.[10]

Experimental

UV spectra were measured in methanol on a Beckman DU-65 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity 300 FT NMR spectrometer with $SiMe_4$ as an internal standard. Elemental analyses were performed on a Perkin-Elmer 240 Elemental Analyzer. TLC was conducted on Merck silica gel F_{254} 60 plates using the following solvent systems (measured by volume): A, chloroform–methanol (9:1); B, toluene–ethanol (4:1). For preparative short-column chromatography Merck TLC gel HF_{254} 60 was used.

Analytical high performance liquid chromatography (HPLC) was performed using the following components from Waters Division of Millipore: Nova Pak C_{18} column (8 × 100 mm Radial-Pak Cartridge), 600E Multisolvent Delivery System with U6K Universal Liquid Chromatography Injector, 486 Tunable Absorbance Detector and 746 Data Module. 9-(2′,3′,-**5′**-tri-*O*-acetyl-β-D-ribofuranosyl)-*N*^{2}-acetylguanine **1** was prepared according to Reese and Saffhill,[21] and then precipitated from chloroform to diethyl ether to get **1** as a white powder. 9-[(2-Acetoxyethoxy)methyl]-*N*^{2}-acetylguanine and its 7-regioisomer (compounds **8** and **3**, respectively), applied as the HPLC and TLC standards, were synthesised as described previously.[3]

7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-*N*^{2}-acetylguanine 6

Tetraacetylguanosine **1** (2.0 g, 4.43 mmol) was heated in an open flask without solvents at 220 °C (temperature of an oil bath) for 10 min. The resulting dark melt was dissolved in toluene–ethanol (6:1, v/v) and chromatographed on a silica gel short column (5 × 9 cm) in this solvent system. Evaporation of fractions 20–25 (*ca.* 20 cm^3) yielded chromatographically pure **6** as an oil. The oil was redissolved in chloroform (5 cm^3), and added dropwise to a stirred mixture of diethyl ether–hexane (300 cm^3; 1:1, v/v). The white precipitate was collected by filtration, washed with hexane and dried under reduced pressure over P_2O_5 to yield compound **6** as a white powder (0.762 g, 38%). The spectroscopic and analytical data are presented in Tables 1–3.

Fractions 38–52 contained pure **1** and were evaporated to recover the unreacted substrate as an oil (1.180 g, 59%).

Reactions of 9- and 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-*N*^{2}-acetylguanines with 2-acetoxyethyl acetoxymethyl ether

General procedure. The peracetylated ribonucleoside (**1** or **6**,

22.6 mg, 0.05 mmol) and 2-acetoxyethyl acetoxymethyl ether[17] (88 mg, 0.5 mmol) were dissolved in dry chlorobenzene (2 cm^3). Reactions were initiated by injecting a solution of toluene-*p*-sulfonic acid monohydrate (0.95 mg, 0.005 mmol) in acetonitrile (50 mm^3) into the pre-thermostatted reaction medium. The transpurination experiments were carried out at 120 °C with stirring in stoppered flasks immersed in a thermostatted oil bath for 2 h. Aliquots of 10 mm^3 (*ca.* 25 nmol) were withdrawn at suitable time intervals (see Figs. 1 and 2). The samples of reaction mixtures were then evaporated to dryness under diminished pressure at room temp. The residues resulting from evaporation of each sample were dissolved in 50% aq. methanol (200 mm^3) and the solutions obtained were used for a preliminary qualitative TLC analysis in solvents A and B, and for the HPLC quantitative analysis (see below).

Results of the HPLC method were also compared to those obtained in the ^{1}H NMR study. Thus, the transpurination experiments of compounds **1** and **6** were repeated on a 0.05 mmol scale for chosen time intervals (20 and 60 min). The reaction mixtures were then evaporated to dryness, the resulting oils were redissolved in $(CD_3)_2SO$ and directly analysed by ^{1}H NMR spectroscopy. The mole fractions of compounds **1**, **6**, **8** and **3** (estimated by integration) were in good agreement (±4%) with the values calculated from the HPLC method for the same time intervals.

HPLC Analysis. Distribution of products was quantitatively determined by HPLC, applying the samples of reaction mixtures described above. Elution with a methanol–water reversed gradient (from 25–60% of aqueous methanol after 20 min, then 60% aq. methanol for the next 10 min) gave a good separation for compounds **1**, **3**, **6**, **8**. The flow rate was set at 1 cm^3 min^{-1} and UV absorption was measured at 260 nm. The following average ε_{260} values were taken for calculation of mole fractions: 16 000 dm^3 mol^{-1} cm^{-1} for the 9-isomers (compounds **1** and **8**) and 13 300 dm^3 mol^{-1} cm^{-1} for the 7-isomers (compounds **6** and **3**). The assignment of the peaks was performed by comparing the retention times and UV spectra with those of the original samples. Retention times (in order of elution): compound **8**, 9.2; **3**, 10.6; **1**, 23.3 and **6**, 24.9 min. The results are presented in Figs. 1 and 2. In addition to those four compounds, the reaction mixtures contained up to 0.5% of N^2-acetylguanine (retention time 4.3 min), not shown in Figs. 1 or 2.

Acknowledgements

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