A Facile Synthesis of 9-(1,3-Dihydroxy-2-propoxymethyl)guanine (Ganciclovir) from Guanosine

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Dedicated to Professor Maciej Wiewiórowski on the occasion of his 80th birthday

Abstract: The potent and selective antiviral drug ganciclovir (**6**) has been synthesized in two steps via transpurination of fully acetylated guanosine (**1**) in the presence of 1,3-diacetoxy-2-(acetoxymethoxy)propane (**2**), followed by deacetylation in aqueous ammonia. The transpurination reaction also provides valuable side products, tetra-*O*-acetyl-β-D-ribofuranose (**5**) and the 7-regioisomer of triacetylganciclovir (**4**); the latter product can be converted to the desired 9-isomer in a thermal 7 ⇄ 9 isomerization.

Key words: ganciclovir, biological activity, guanosine, transglycosylation, transpurination

9-(1,3-Dihydroxy-2-propoxymethyl)guanine (ganciclovir; DHPG; **6**), one of the most important nucleoside analogs of biological activity, exhibits a selective antiherpetic action[1–4] including clinical efficacy against cytomegalovirus.[5,6] Ganciclovir is readily phoshorylated in infected cells by the HSV thymidine kinase,[7,8] and its 5'-triphosphate acts as a 'chain terminating' nucleotide analog, inhibiting the viral DNA-polymerases. Ganciclovir has also found a promising application in the so-called 'suicide gene' therapy of brain tumors,[9,10] and recently of lung tumors[11] and peritoneal carcinomatosis.[12] Chiral synthesis of a DNA decamer containing 9-(1,3-dihydroxy-2-propoxymethyl)guanine has been reported and effects of incorporation of this acyclonucleoside on thermal stability, duplex structure, and thermodynamics of duplex formation has been investigated.[13,14]

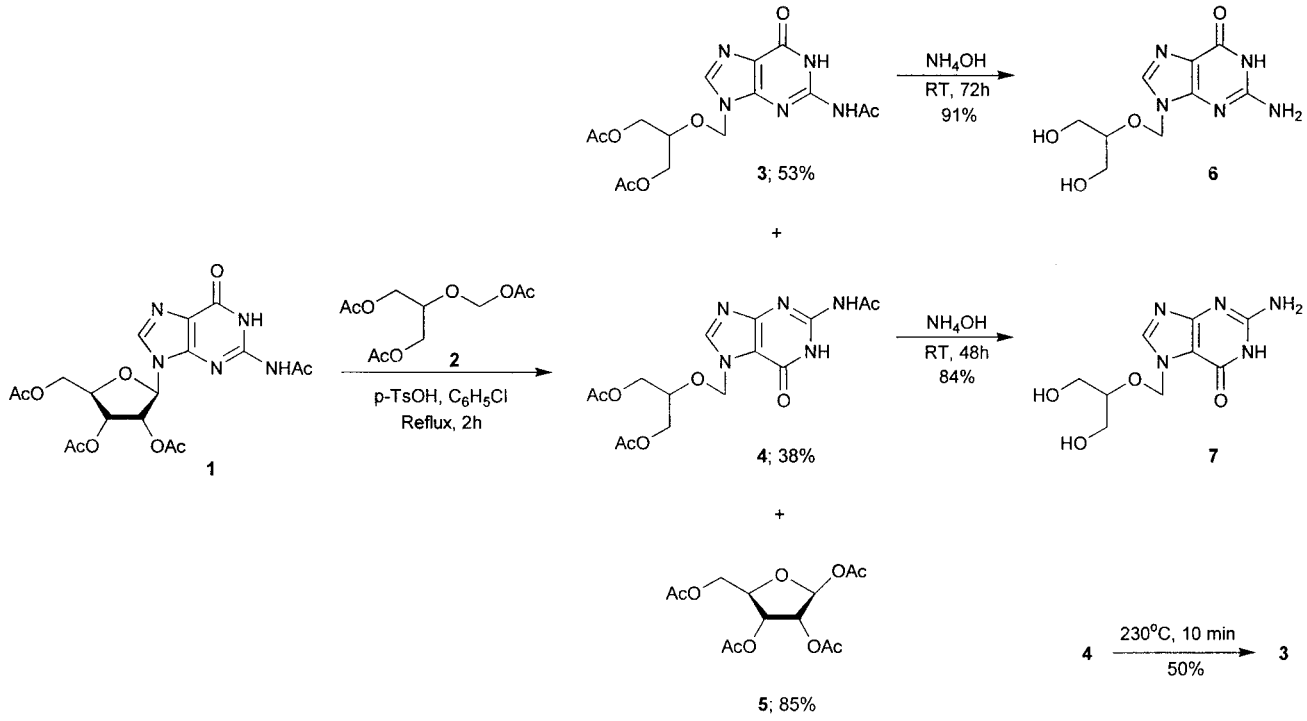
The first chemical syntheses of ganciclovir were reported almost simultaneously by several research groups.[1,3,4] Those syntheses involved glycosylation reactions of N^2-monoacetyl- or 9,N^2-diacetylguanine with chloromethyl or acetoxymethyl derivatives of glycerol. All those procedures suffered from some practical limitations, i.e. low overall yields, lack of regioselectivity usually observed in alkylation of guanine,[15–17] and difficulties in separation of the resulting 7- and 9-regioisomers of the product. On the other hand, the use of tetrabutylammonium fluoride in alkylation of silylated guanine reportedly resulted in the formation of a pure 9-regioisomer of ganciclovir.[18] Besides, due to the use of benzyl groups for protection of hydroxyl functions, some synthetic methods[1,4,18,19] required an additional step in the final deblocking of the product, catalytic hydrogenolysis over palladium. This further reduced the already low yield of synthesis and considerably limited a scale-up perspective. More recently, the yield of synthesis of **6** has been slightly improved in the reaction of diacetylguanine with 1,3-dipivaloyloxy-2-[(methylsulfinyl)methoxy]propane.[20] In this method, however, preparation of the pseudosugar component is rather laborious due to three steps of synthesis, foul-smelling byproducts, and a moderate overall yield (29% from glycerol). Ganciclovir can also be obtained from 5-aminoimidazole-4-carboxamide (AICA) by applying a modified Yamazaki ring closure reaction in a 3-step synthesis with an overall yield of 3%.[21]

We now wish to report an efficient synthetic route to ganciclovir (**6**) from inexpensive and readily accessible guanosine, using one kind of protection, acetyl groups, for all reactive functions of substrates. The acyclic sugar analog was prepared in reaction of 1,3-dichloro-2-(chloromethoxy)propane[22,23] with an excess of potassium acetate, which gave 1,3-diacetoxy-2-(acetoxy-methoxy)-propane (**2**) in 71% yield. This compound may also be obtained in two steps from 4-chloromethyl-1,3-dioxolane.[3]

The main synthetic step of our procedure, transpurination of tetraacetylguanosine (**1**)[24] in chlorobenzene with the pseudosugar component **2**, performed in the presence of *p*-toluenesulfonic acid, gave a mixture of products: triacetylganciclovir (**3**), its 7-regioisomer **4**, and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (**5**). These products were easily separated using silica gel chromatography, in 53, 38 and 85% yields, respectively (Scheme). The mechanism of glycosyl exchange reactions in the guanine nucleosides series has been discussed recently.[17,25]

The side product formed in this approach, 7-regioisomer **4** was then converted to the desired product **3** in the reaction of thermal 7 ⇄ 9 isomerization. Thus, heating of **4** at 230 °C for 10 min resulted in a mixture of the regioisomers, from which the 9-substituted compound **3** was isolated in 50% yield. Finally, the triacetyl derivative **3** was deprotected in aqueous ammonia to afford ganciclovir (**6**) in an almost quantitative yield. In a similar way, deblocking of **4** gave the 7-regioisomer of ganciclovir (**7**).

Products **3–7** were obtained in a crystalline state. Their structures and purity were proven by ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), ultraviolet spectra, thin-layer chromatography and elemental analysis. The method presented here appears to be the simplest and the most efficient approach to the synthesis of ganciclovir. Moreover, guanosine serves not only as a purine donor, but also as a substrate for synthesis of tetraacetylribose (**5**) - a valuable component in the nucleoside chemistry.



Scheme

9-(2',3',5'-Tri-*O*-acetyl-β-D-ribofuranosyl-N^2-acetylguanine (**1**) was prepared according to Reese and Saffhill,[25] and then precipitated by addition of its solution in $CHCl_3$ to Et_2O giving **1** as a white powder. 1,3-Dichloro-2-(chloromethoxy)propane was obtained from 1,3-dichloropropan-2-ol.[22,23] Melting points: Laboratory Devices Mel-Temp II micromelting point apparatus (uncorrected). UV spectra: Beckman DU-65 spectrophotometer. NMR spectra: Varian Unity 300 FT NMR spectrometer (^1H: 300 MHz; ^{13}C: 75 MHz). Chemical shifts, δ, are expressed in ppm downfield from TMS. Elemental analyses: Perkin-Elmer 240 Elemental Analyzer; satisfactory microanalyses were obtained for **3–7**: C ± 0.15%, H ± 0.09%, N ± 0.22%. TLC: Merck silica gel F_{254} 60 plates, in the following solvent systems (v/v): A, $CHCl_3$/MeOH (9:1); B, toluene/EtOH (4:1); C, *i*-PrOH/concd NH_4OH/H_2O (7:1:2). Preparative short-column chromatography: Merck TLC gel 60.

1,3-Diacetoxy-2-(acetoxymethoxy)propane (2)

A suspension of anhyd potassium acetate (50 g, 0.51 mol) in DMF (200 mL) was concentrated under reduced pressure to a volume of ca. 170 mL and then 1,3-dichloro-2-(chloromethoxy)propane[22,23] (15.0 g, 84.5 mmol) was added. The suspension was refluxed with vigorous stirring for 2 h. After cooling, the mixture was diluted with H_2O (500 mL) and extracted with $CHCl_3$ (3 x 250 mL) The combined extracts were washed with sat. $NaHCO_3$ (200 mL), dried ($MgSO_4$) and concentrated on a rotary evaporator at 5 mbar, 40 °C. Distillation of the crude product (129–130 °C/0.5 mbar) gave **2** as a colorless syrup. Yield 13.88 g (71%).

^1H NMR (300 MHz, $CDCl_3$): δ = 2.08 (s, 6H, CH_3CO), 2.11 (s, 3H, CH_3CO), 4.16 (m, 5H, CH_2CCH_2), 5.34 (s, 2H, OCH_2O).

9-(1,3-Diacetoxy-2-propoxymethyl)-N^2-acetylguanine (3)

Method A. A mixture of **1** (10.0 g, 22.15 mmol), **2** (11.0 g, 47.39 mmol), and *p*-toluenesulfonic acid monohydrate (0.063 g, 3.323 mmol) was refluxed with stirring in anhyd chlorobenzene (100 mL)

Table 1 Chemical Shifts (TMS, δ) in ^1H NMR Spectra

Compound	N^2H	N^1H	8-H	NCH_2O	OH	CH	CCH_2	$NCOCH_3$	$OCOCH_3$
3[a]	12.01	9.51	7.82	5.53	–	4.15		2.34	2.02
	bs, 1	bs, 1	s, 1	s, 2		m, 5		s, 3	s, 6
4[a]	12.47	11.22	7.99	5.87	–	4.09		2.44	2.01 2.00
	bs,1	bs, 1	s, 1	s, 2		m, 5		s, 4	2s, 6
6[b]	6.50	10.64	7.81	5.44	4.62	3.55	3.38	–	–
	bs, 2	bs, 1	s, 1	s, 2	t, 2	p, 1	m, 4		
7[b]	6.19	10.84	8.09	5.65	4.59	3.51	3.37	–	–
	bs, 2	bs, 1	s, 1	s, 2	t, 2	p, 1	m, 4		

[a] Recorded in $CDCl_3$.
[b] Recorded in DMSO-d_6.

Table 2 Chemical Shifts (TMS, δ) in ^{13}C NMR Spectra

Compound	N-Ac	O-Ac	C6	C2	C4	C8	C5	CH	NCH_2	CCH_2	N-Ac	O-Ac
3[a]	171.94	170.93	155.50	147.91	148.69	138.93	121.07	74.44	72.14	63.01	24.34	20.81
4[a]	173.35	170.49	156.86	148.38	153.20	143.85	111.86	74.76	74.94	63.09	24.68	20.68
6[b]	–	–	156.71	153.69	151.18	137.55	116.33	79.91	71.39	60.76	–	–
7[b]	–	–	160.16	152.81	154.41	143.75	107.62	79.79	74.31	60.74	–	–

[a] Recorded in $CDCl_3$.
[b] Recorded in DMSO-d_6.

for 2 h. The solvent was then removed under reduced pressure. A resulting oil was redissolved in $CHCl_3/CH_3OH$ (9:1) and adsorbed on a portion of silica gel (25 g, 70–230 mesh) by evaporation. The dried gel was applied on a silica gel short column (7 x 11 cm). Products were eluted with a toluene/EtOH gradient (from 95:5 to 4:1, respectively), and 20 mL fractions were collected. Fractions # 13–26 contained tetraacetylribose **5**, and fractions # 48–66 - the 7-regioisomer **4**. Evaporation of fractions # 76–110 gave homogenous by TLC compound **3** as an oil. An analytical sample was crystallized from EtOH, mp 175 °C. Yield 4.15 g (53%), R_F 0.58 (A), 0.21 (B).

λ_{max} (MeOH) = 259 nm (log ε 4.21), 278 (4.06).

Method B. The 7-regioisomer **4** (3.0 g, 7.87 mmol) obtained as a side product in Method A was heated in an open flask at 230 °C for 10 min. The resulting mixture of the isomers **3** and **4** was separated by short-column chromatography as it was described in Method A. Evaporation of the appropriate fractions allowed the recovery of the starting material (**4**) as a crystallizing oil (1.33 g, 44%). Further fractions contained the desired 9-regioisomer (**3**) as an oil after evaporation of solvents. Yield 1.49 g (50%). The product was in all respects (^1H and ^{13}C NMR, UV, TLC) identical to that obtained in Method A.

7-(1,3-Diacetoxy-2-propoxymethyl)-*N*^{2}-acetylguanine (4)
Evaporation of fractions containing the 7-regioisomer **4**, obtained in chromatography after the reaction of **1** and **2**, gave pure product **4** as a crystallizing oil. Yield 3.18 g (38%). An analytical sample was recrystallized from toluene/EtOH (4:1), mp 187.5 °C, R_F 0.63 (A), 0.40 (B).

λ_{max} (MeOH) = 262 nm (log ε 4.13), 280 (sh; 4.01).

1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose (5)
The first fractions obtained in chromatography after the reaction of **1** and **2** contained tetraacetylribose (**5**). The fractions were pooled and evaporated to an oil, which was redissolved in diisopropyl ether on heating. The yellow solution was decolorized by refluxing in the presence of activated charcoal, and after filtration crystallized from this solution, mp 81.5 °C. Yield 6.03 g (85%). This product was in all respects identical with an authentic sample of **5**,[27] R_F 0.95 (A), 0.73 (B).

^1H NMR (300 MHz, $CDCl_3$): δ = 2.08, 2.10, 2.11, 2.13 (4s, 12H, CH_3CO), 4.17 (dd, 1H, 5b-H), 4.37 (m, 2H, 4-H, 5a-H), 5.35 (m, 2H, 2-H, 3-H), 6.17 (s, 1H, 1-H).

9-(1,3-Dihydroxy-2-propoxymethyl)guanine (6)
A sample of the fully acetylated derivative **3** (3.81 g, 10.00 mmol) was dissolved in 27% NH_4OH (120 mL) and stirred at r.t. for 3 days. After this time the solvent was gently evaporated under diminished pressure and the obtained white solid was crystallized from 80% EtOH, mp 246 °C (dec). Yield 2.32 g (91%), R_F 0.04 (A), 0.48 (C).

λ_{max} (H_2O) 254 nm (log ε 4.15), 270 (sh; 4.05).

7-(1,3-Dihydroxy-2-propoxymethyl)guanine (7)
A solution of the fully acetylated 7-regioisomer **4** (0.114 g, 0.30 mmol) in 27% NH_4OH (5 mL) was stirred at r.t. for 2 days. The solution was then concentrated to a volume of ca. 3 mL and left aside at 5 °C. A resulting crystalline material was collected by filtration, washed with acetone and dried in vacuo, mp >300 °C (darkened >258 °C). Yield 0.064 g (84%), R_F 0.06 (A), 0.50 (C).

λ_{max} (H_2O) = 244 nm (log ε 4.10), 286 (4.17).

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