Synthesis of Acyclowyosine and Acyclo-3-methylguanosine, as Probes for some Chemical and Biological Properties resulting from the N-3 Substitution of Guanosine and its Analogues

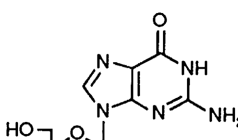
Bozenna Golankiewicz,*,[a] Tomasz Ostrowski,[a] Jerzy Boryski[a] and Erik De Clercq[b]
[a] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland*
[b] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

Acyclo analogues of wyosine **2** and 3-methylguanosine **3**, *viz.* 9-[(2-hydroxyethoxy)methyl]-3-methyl-1, *N*-2-(prop-1-ene-1,2-diyl)guanine **5a** and 9-[(2-hydroxyethoxy)methyl]-3-methylguanine (3-methylacyclovir, **6a**) were synthesized from acyclovir **4**. The route to compound **5a** involved methylation of the tricyclic acetate **7b** with diazomethane–zinc iodide reagent and subsequent deacetylation; tricycle **5a** was transformed into compound **6a** with *N*-bromosuccinimide followed by ammonium hydroxide. Direct coupling of 3-methylguanine with the appropriate chain component resulted exclusively in formation of compound **9a**, the *N*-7 regioisomer of **6a**. The glycosidic hydrolysis rates of compounds **2** and **3** differed from those of the much less sterically compressed analogues **5a** and **6a** by less than one order of magnitude. This contrasts with the 10^5-fold increase in hydrolysis rate of compounds **2** and **3**, compared with that of guanosine **1**, and suggests that electronic factors must play an important role in the accelerated hydrolysis of 9-substituted 3-methylguanine derivatives. The antiviral activity of acyclovir was virtually abolished following N-3 methylation (**6a**). The other compounds (**2**, **3** and **5a**) also failed to show any antiviral activity.

The glycosidic bond of the tricyclic derivatives of guanosine **1**, called Y nucleosides, occurring in $tRNA^{Phe}$ is cleaved with exceptional ease under mildly acidic conditions.[1] According to the kinetic experiments of Itaya *et al.*, the rate of hydrolysis of the simplest member of the family, wyosine† [3-methyl-1,*N*-2-(prop-1-ene-1,2-diyl)guanosine **2**] is five orders of magnitude higher than that of guanosine **1**.[2b] 3-Methylguanosine **3** behaves similarly to **2**.[2]

The acceleration of the dissociation has been ascribed to the steric repulsion between the ribose and the heterocyclic base moiety induced by the position of the methyl group in both compounds **2** and **3**.[2a,3] This explanation has recently been questioned in as far as there exists a nonconstrained conformation and electronic factors have been proposed as a rationale for the accelerated hydrolysis.[4]

To evaluate the contribution of both factors, steric and electronic, we synthesized, and subjected to acidic hydrolysis, two model compounds: 9-[(2-hydroxyethoxy)methyl]-3-methyl-1,*N*-2-(prop-1-ene-1,2-diyl)guanine **5a** and 9-[(2-hydroxyethoxy)methyl]-3-methylguanine **6a**. In these 'acyclo' analogues of compounds **2** and **3** characteristic electronic features of the 9-substituted 3-methylguanine system are conserved but steric compression is relieved.



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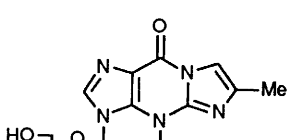

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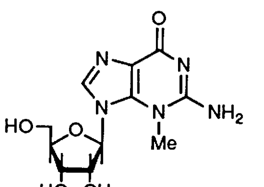

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From another viewpoint, compounds **5a** and **6a** are derivatives of the potent antiviral agent 9-[(2-hydroxy-ethoxy)methyl]guanine (acyclovir, **4**). In continuation of our study of the role of particular nitrogen centres in the antiviral activity of acyclovir,[5] we examined the biological activity of compounds **5a** and **6a** as well as that of their parent ribosides **2** and **3**.

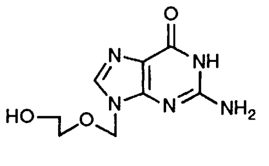
Results and Discussion

Synthesis.—The acyclovir analogues **5a** and **6a** were obtained from compound **4** *via* 9-[(2-hydroxyethoxy)methyl]-1,*N*-2-(prop-1-ene-1,2-diyl)guanine **7a**,[5] using modifications of the procedures which we previously reported for the parent β-D-ribosides **2**[6] and **3**.[7]

Treatment of compound **7a** with acetic anhydride in pyridine provided the *N*,*O*-diacetyl derivative, which was then partly deblocked with a mixture of pyridine–methanol–water (1:1:1) to afford 9-[(2-acetoxyethoxy)methyl]-1,*N*-2-(prop-1-ene-1,2-diyl)guanine **7b** in 95% yield. Methylation of compound **7b** with diazomethane in the presence of zinc iodide[8] in a mixture of diethyl ether–dichloromethane–dimethylformamide (DMF) solution gave fluorescent (purine-3)-methyl derivative **5b** in 34% yield. The latter was deacetylated with aqueous ammonia in methanol to provide crystalline 9-[(2-hydroxyethoxy)-methyl]-3-methyl-1,*N*-2-(prop-1-ene-1,2-diyl)guanine (acyclo-wyosine, **5a**) in 90% yield. Reaction of compound **5a** with *N*-

† Throughout this paper the nomenclature and numbering of the tricyclic compounds are in purine convention to underline their relation to guanosine. The IUPAC name for wyosine is: 4,9-dihydro-4,6-dimethyl-9-oxo-3 (β-D-ribofuranosyl)-3*H*-imidazo[1,2-*a*]purine.

bromosuccinimide (NBS) at pH 4.8 followed by treatment with aqueous ammonia resulted in removal of the isopropene group to produce crude 9-[(2-hydroxyethoxy)methyl]-3-methylguanine (acyclo-3-methylguanosine, 3-methylacyclovir, **6a**). Chromatographic purification in the form of diacetyl derivative **6b** and deblocking with methanolic ammonia gave pure, crystalline compound **6a** in 39% yield.



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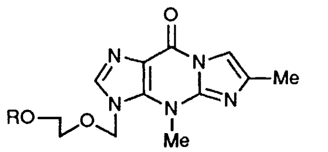
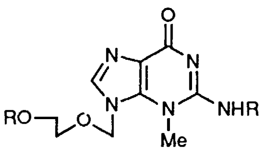


5a; R = H
b; R = Ac



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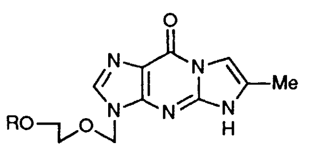
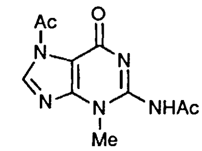
b; R = Ac



7a; R = H
b; R = Ac



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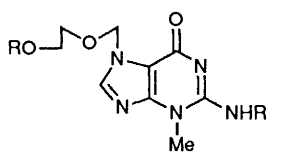
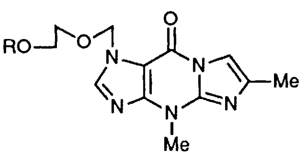
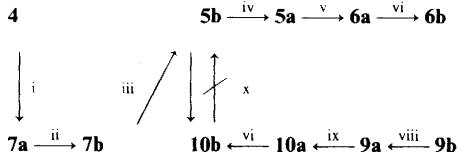


9 a; R = H
b; R = Ac



10 a; R = H
b; R = Ac

Examination of molecular models showed pronounced reduction of possible steric interactions between substituents at N-9 and N-3 when going from 9-(β-D-ribofuranosyl) (**2** and **3**), to 9-[(2-hydroxyethoxy)methyl] (**5a** and **6a**), derivatives. However, there was no effect on the regioselectivity of coupling of 3-methylguanine with an appropriate chain component in place of a ribosyl one. Analogously to ribosylation of 3-methylguanine,[9] direct introduction of the (2-hydroxyethoxy)methyl unit took place exclusively at the N-7 position. Thus, *N*-7-substituted isomers of acyclo-3-methylguanosine and acyclowyosine were obtained as follows. *N*-2,7-Diacetyl-3-methylguanine **8** was treated with 2-(acetoxymethoxy)ethyl acetate in toluene in the presence of toluene-*p*-sulphonic acid (PTSA). After chromatography on silica gel, crystalline 7-[(2-acetoxyethoxy)methyl]-*N*-2-acetyl-3-methylguanine **9b** was separated in 69% yield. Acetyl groups were removed by 33% aqueous diethylamine to give the alcohol **9a** in 98% yield. Treatment of compound **9a** in DMF with potassium carbonate, followed by bromoacetone, produced tricyclic, fluorescent product **10a** in 29% yield after chromatographic separation. Acetylation of compound **10a** with acetic anhydride in pyridine provided the acetate **10b** in 43% yield. The latter compound could not be isomerized thermally into its 9-substituted congener **5b**. Only the reverse transformation **9** ⟶ **7** (**5b** ⟶ **10b**) occurred, similarly to transglycosylation noted



Scheme 1 *Reagents and conditions*: i, NaH, Me_2SO, room temp.; then $BrCH_2COMe$; ii, Ac_2O, pyridine, room temp.; then pyridine–aq. MeOH; iii, CH_2N_2, ZnI_2, CH_2Cl_2–Et_2O–DMF; iv, NH_4OH; v, NBS, buffer (pH 4.8); then NH_4OH; vi, Ac_2O, pyridine, room temp.; vii, $AcOCH_2CH_2OCH_2OAc$, PTSA, toluene, reflux; viii, 33% aq. Et_2NH, reflux; ix, K_2CO_3, DMF, room temp.; then $BrCH_2COMe$; x, 200–250 °C

previously for fully acetylated wyosine[10] and 3-methylguanosine.[9]

Spectral and Chromatographic Properties.—The regioisomeric *N*-7 and *N*-9 acyclonucleosides bearing a 3-methylguanine structural unit exhibit characteristic diagnostic differences in their ^{1}H NMR spectra (Table 1). In accord with observations reported for a series of *N*-7- and *N*-9-alkylated purines[11] the 8-H proton signals of the *N*-9-isomers **5** and **6** are shifted upfield relative to the corresponding 8-H signals of the *N*-7-isomers **10** and **9**. In addition, 3-Me proton signals are shifted downfield for the *N*-9-isomers relative to the corresponding resonances of the *N*-7-isomers.

In agreement with previous data[12,13] on various guanine nucleosides and their analogues, *N*-7-isomers of novel acyclonucleosides are more mobile than their *N*-9-counterparts when analysed by normal-phase TLC on silica gel (Table 2).

As expected, the ultraviolet absorption patterns of acyclonucleosides (Table 2) closely resemble those of the corresponding ribosides.

Kinetics of Acid-catalysed Hydrolysis.—The results of the hydrolysis measurements are summarized in Table 3. As already mentioned, examination of molecular models showed a pronounced reduction of possible steric interactions between substituents at N-9 and N-3 when going from 9-(β-D-ribofuranosyl) (**2** and **3**), to 9-[(2-hydroxyethoxy)methyl] (**5a** and **6a**), derivatives. The pseudo-first-order rate constants for hydrolysis of the glycosidic bonds, however, were lower by a factor of *ca.* 30 for the pair **2**/**5a**, and by *ca.* 20 for the pair **3**/**6a**. It should also be taken into account that replacement of the ribofuranosyl substituent with the (2-hydroxyethoxy)methyl group reduces the hydrolysis rate by a factor of seven for the pair guanosine **1** and acycloguanosine **4**.

We interpret out kinetic data as well as previously reported data[2] as indicating that electronic features of 3-methylguanosine-type nucleosides play an important role in their rapid acidic hydrolysis. Indeed, introduction at position N-9 of a smaller substituent retards hydrolysis, whereas introduction of a more bulky one at N-3 enhances the hydrolysis rate. The changes in hydrolysis rate, however, are not higher than one order of magnitude and are thus of relatively minor importance compared with the change in hydrolysis rate (five orders of magnitude) when the guanosine derivatives are compared with their 3-methyl derivatives.

Biological Activity.—The two novel acyclic nucleosides analogues prepared in this study (**5a** and **6a**) together with their β-D-ribofuranosyl parents (**2** and **3**) were evaluated for their antiviral activity in a wide variety of assay systems: herpes simplex virus type 1 (strains KOS, F, McIntyre), herpes simplex virus type 2 (strains G, 196, Lyons), thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains B2006, VMW18), vaccinia virus and vesicular stomatitis virus in primary rabbit kidney (PRK) cells; vesicular stomatitis virus, poliovirus type 1

Table 1 ^1H NMR spectra (δ)[a] of acyclowyosine, acyclo-3-methylguanosine and related compounds

Compound	Group N-2-H	8-H	NCH_2O	OH	CH_2CH_2	Prop-1-ene-1,2-diyl	Me	Ac
7a[b]	12.42 (1, br s)	8.03 (1, s)	5.49 (2, s)	4.69 (1, br s)	3.50 (4, br s)	7.36 (1, d), 2.27 (3, d)		
5a		8.00 (1, s)	5.76 (2, s)	4.70 (1, br t)	3.50 (4, 2 s)	7.39 (1, d), 2.22 (3, d)	4.09 (3, s)	
6a	6.91 (2, br s)	7.74 (1, s)	5.63 (2, s)	*n*	3.47 (4, s)		3.69 (3, s)	
9a	6.93 (2, br s)	8.07 (1, s)	5.69 (2, s)	4.67 (1, t)	3.49 (4, s)		3.53 (3, s)	
10a[c]		8.27 (1, s)	5.84 (2, s)	4.86 (1, s)	3.65 (4, s)	7.39 (1, d), 2.31 (3, d)	3.90 (3, s)	
7b	12.43 (1, br s)	8.02 (1, s)	5.49 (2, s)		4.06, 3.73 (4, 2 m)	7.37 (1, d), 2.27 (3, d)		1.95 (3, s)
5b		8.01 (1, s)	5.78 (2, s)		4.11, 3.69 (4, 2 m)	7.39 (1, d), 2.23 (3, d)	4.07 (3, s)	1.86 (3, s)
6b	*n*	8.09 (1, s)	5.76 (2, s)		4.11, 3.63 (4, 2 m)		3.88 (3, s)	2.13, 1.93 (6, 2 s)
9b	13.59 (1, br s)	8.43 (1, s)	5.70 (2, s)		3.77, 3.64 (4, 2 m)		3.62 (3, s)	2.13, 1.95 (6, 2 s)
10b		8.44 (1, s)	5.76 (2, s)		4.07, 3.73 (4, 2 m)	7.38 (1, d), 2.23 (3, d)	3.81 (3, s)	1.94 (3, s)

[a] Recorded at 90 Mz in $(CD_3)_2SO$ solution unless otherwise stated; referred to Me_4Si. Signals are designated as *n* if no firm assignment could be made. Figures preceding the observed multiplicities are the numbers of protons as estimated by integration. [b] Data from ref. 5. [c] In CD_3OD.

Table 2 UV spectral data and TLC chromatography R_f-values of acyclowyosine, acyclo-3-methylguanosine and related compounds

Compound	λ_{max} (water)/nm (ε/dm^3 mol^{-1} cm^{-1})	R_f-value (× 100) in system[a] A	B	C
5a	237 (27 800), 296 (6500)	54	02	39
6a	217 (16 400), 248sh (5500), 266 (6400)	33	00	00
9a	215 (14 400), 237sh (6500), 271 (6800)	33	00	11
10a	233 (20 600), 264 (4200), 312 (4300)	57	25	69
5b	236 (25 800), 296 (7400)	63	14	77
6b	218 (13 900), 272 (8500)	59	10	70
9b	227 (10 700), 270 (7900)	60	45	86
10b	233 (21 300), 265 (3900), 315 (4200)	65	47	93
7b	231 (26 200), 285 (8800)	73	13	77

[a] Bu^nOH–AcOH–water (5:3:2); B, $CHCl_3$–MeOH (95:5); C, $CHCl_3$–MeOH (4:1).

Table 3 Apparent first-order rate constants for glycosidic hydrolysis of 9-(β-D-ribofuranosyl)- and 9-[(2-hydroxyethoxy)methyl]guanine derivatives

Compound	k_{obs}/min^{-1} 0.1 mol dm^{-3} HCl; 25 °C	pH 2.9, 37 °C
2	4.4×10^{-1} [a]	1.3×10^{-2}
5a	1.5×10^{-2}	9.5×10^{-4}
3	9.8×10^{-1} [a]	3.3×10^{-2}
6a	5.3×10^{-2}	3.5×10^{-3}
1	5.7×10^{-6} [a]	
4	8.1×10^{-7} [b]	

[a] Rate constants taken from the literature (ref. 2). [b] Calculated from the Arrhenius equation following measurements done at 65, 85 and 95 °C.

and Coxsackie B4 virus in HeLa cells; parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus and Semliki forest virus in Vero cells; cytomegalovirus (strains AD-169, Davis), varicella-zoster virus (strain OKA) and TK^- varicella-zoster virus (strains YSR, 07-1) in human embryonic lung (HEL) cells. Acyclovir **4** was included as the reference material. Acyclovir was active against the herpes simplex virus (strains KOS, F, McIntyre, G, 196, Lyons) at a minimum inhibitory concentration (MIC) of 0.01–0.1 μg cm^{-3}; its MIC for varicella zoster virus (strain OKA) was 1 μg cm^{-3}. With compounds **2**, **3**, **5a** and **6a**, no antiviral activity was noted in any system (at concentrations up to 400 μg cm^{-3}), except for compound **6a** which showed an MIC of 10 μg cm^{-3} against varicella-zoster virus (strain OKA).

When examining several mono-, di- and tri-*N*-substituted derivatives of acyclovir **4** for their antiviral activity, we found that methylation at N-2 annihilated the antiviral activity of compound **4**, whereas methylation at N-7 only reduced it. The N-1 position did not appear important in this respect, since 1-methylacyclovir showed considerable antiviral activity. So did the tricyclic compound **7a**, in which N-1 and N-2 are blocked by a 1,*N*-2-(prop-1-ene-1,2-diyl) linkage. None of the *N*-3-substituted derivatives of acyclovir, compounds **5a**, **6a**, **2** and **3**, showed appreciable antiviral activity, as mentioned above. This points to the significance of the N-3 position in the biological activity of acyclovir. In 3-methylacyclovir **6a**, two nitrogen centres (exocyclic N-2 amino and N-7), which are crucial for antiviral activity, were conserved. Yet, the compound was virtually inactive. In summary, the antiviral data reported here and previously[5] indicate the following order of (decreasing) importance in the antiviral activity of acyclovir: N-3 $\geqslant$ N-2 > N-7 > N-1.

Experimental

M.p.s were determined in open capillaries in a micromelting point apparatus and are uncorrected. UV spectra were measured by a Zeiss Specord UV-vis and a Zeiss VSU-2P spectrophotometer. ^1H NMR spectra were recorded on a JEOL FX 90Q FT NMR spectrometer. TLC was conducted on Merck precoated silica gel plates F_{254} Type 60 with the following solvent systems (measured by volume): A, butan-1-ol–glacial acetic acid–water (5:3:2); B, chloroform–methanol (95:5); C, chloroform–methanol (4:1). For preparative short-column chromatography, Merck TLC gel HF_{254} Type 60 was used. Elemental analyses were performed on a Perkin-Elmer 240 elemental analyser and Hewlett-Packard 185 CHN analyser. Determination of the hydrolysis rates was based on UV absorption measurements, following a previously reported procedure.[14] Samples of wyosine **2**, 3-methylguanosine **3** and 9-[(2-hydroxyethoxy)methyl]-1,*N*-2-(prop-1-ene-1,2-diyl)-guanine **7a** were prepared according to refs. 6, 7 and 5, respectively.

9-[(2-*Acetoxyethoxy*)*methyl*]-1,*N*-2-(*prop*-1-*ene*-1,2-*diyl*)-*guanine* **7b**.—An anhydrous suspension of the alcohol **7a** (1.843 g, 7.0 mmol) in pyridine (25 cm^3) was stirred with acetic anhydride (5.3 cm^3, 56 mmol) at room temperature for 2.5 h. The resulting clear solution was evaporated to dryness and the oily residue was redissolved in pyridine–methanol–water (1:1:1; 40 cm^3) to remove the 2-NH-acetyl group. After 5 h at room temperature, TLC in solvent B showed complete conversion into the monoacetylated product **7b**. The reaction mixture was evaporated to dryness, and co-evaporated with toluene and then with chloroform to give **7b** as a solid (2.09 g, 98%), m.p. 198–200 °C (Found: C, 50.8; H, 5.1; N, 22.1. $C_{13}H_{15}N_5O_4 \cdot 0.25H_2O$ requires C, 50.4; H, 5.0; N, 22.6%).

9-[(2-*Acetoxyethoxy*)*methyl*]-3-*methyl*-1,*N*-2-(*prop*-1-*ene*-1,2-*diyl*)*guanine* **5b**.—A solution of zinc iodide (4.79 g, 15.0 mmol) in diethyl ether (50 cm^3) was titrated with an ethereal solution of diazomethane until no further decolourization of the resultant suspension occurred. To the suspension of this methylating reagent was added a solution of the acetate **7b** (305 mg, 1.0 mmol) in a mixture of dichloromethane and DMF (5:1; 30 cm^3). After being stirred for 45 min at room temperature, the reaction mixture was treated with 1 mol dm^3 aq. ammonium hydrogen carbonate (50 cm^3) and a white precipitate was filtered off. The organic phase was separated and the aqueous phase of the filtrate was extracted with chloroform several times. The combined organic layers were washed successively with a small amount of 0.1 mol dm^{-3} aq. sodium thiosulphate and water, then dried over sodium sulphate and evaporated to dryness. The residual oil was chromatographed on a silica gel column (3.5 × 15 cm) in chloroform–methanol (95:5), and 15 cm^3 fractions were collected. Fractions 13–15 contained 3,7-dimethyl-1,*N*-2-(prop-1-ene-1,2-diyl)guanine (28 mg, 13%). Evaporation of fractions 25–29 yielded chromatographically pure *compound* **5b** (107 mg, 34%) as a solid. An analytical sample was recrystallized from methanol, m.p. 178–180 °C (Found: C, 52.55; H, 5.0; N, 22.0. $C_{14}H_{17}N_5O_4$ requires C, 52.7; H, 5.4; N, 21.9%).

9-[(2-*Hydroxyethoxy*)*methyl*]-3-*methyl*-1,*N*-2-(*prop*-1-*ene*-1,2-*diyl*)*guanine* (*Acyclowyosine*, **5a**).—Compound **5b** (400 mg, 1.26 mmol) was dissolved in methanol (40 cm^3) and the solution was treated with 25% aq. ammonia (40 cm^3). After 24 h at room temperature the crystalline *product* **5a** was collected by filtration and dried under diminished pressure over phosphorus pentaoxide. Concentration of the filtrate gave another crop of crystals; total yield 314 mg (90%), m.p. 187–188 °C (Found: C, 48.8; H, 6.0; N, 23.7. $C_{12}H_{15}N_5O_3 \cdot H_2O$ requires C, 48.8; H, 5.8; N, 23.7%).

9-[(2-*Hydroxyethoxy*)*methyl*]-3-*methylguanine* (*Acyclo*-3-*methylguanosine*, 3-*Methylacyclovir*, **6a**).—NBS (96 mg, 0.54 mmol) was added to a suspension of the bicycle **5a** (84 mg, 0.3 mmol) in 0.5 mol dm^{-3} acetate buffer, pH 4.8 (5 cm^3). The suspension was stirred at room temperature for 90 min then treated with 25% aq. ammonia (5 cm^3) and stirred again for 30 min. After this time, TLC in solvent A showed the presence of a small amount of unchanged substrate **5a** and product **6a**. The solution was carefully evaporated to dryness and the residue after evaporation was redissolved in pyridine (1.5 cm^3) containing acetic anhydride (0.5 cm^3, 5.3 mmol). The resulting mixture was stirred for 2.5 h, then evaporated to afford an oil, which was chromatographed on a silica gel column (2.8 × 15 cm) with a chloroform–methanol gradient (from 95:5 to 9:1, respectively). The first fractions, 19–22, contained compound **5b** in form of an oil (15 mg, 15%) after evaporation. Evaporation of fractions 23–32 gave the TLC-homogeneous product **6b** (84%) as an oil. This product, without further purification, was deprotected by treatment with a mixture of methanol–25% aq. ammonia (1:1; 10 cm^3) for 24 h. The resulting solution was evaporated and the residue obtained after evaporation was recrystallized from water, giving the *title compound* **6a** (27 mg, 38% from **5a**), m.p. 213–215 °C (Found: C, 43.9; H, 5.5; N, 28.0. $C_9H_{13}N_5O_3 \cdot 0.5H_2O$ requires C, 43.55; H, 5.7; N, 28.2%).

N-2,7-*Diacetyl*-3-*methylguanine* **8**.—3-Methylguanine (165 mg, 1.0 mmol) was heated with acetic anhydride (4.0 cm^3, 42.4 mmol) under reflux for 3 h. The acetylated product **8** was precipitated by addition of diethyl ether (30 cm^3), filtered off and dried (150 mg, 60%), m.p. >300 °C; δ_H [90 MHz; $(CD_3)_2SO$; Me_4Si] 2.15 (3 H, s, 2-NH*Ac*), 2.90 (3 H, s, 7-Ac) 3.64 (3 H, s, NMe), 8.84 (1 H, s, 8-H) and 13.46 (1 H, s, NH).

N-2-*Acetyl*-7-[(2-*acetoxyethoxy*)*methyl*]-3-*methylguanine* **9b**.—2-(Acetoxymethoxy)ethyl acetate (180 mg, 1.03 mmol) and PTSA monohydrate (4 mg, 0.02 cm^3) were added to a suspension of compound **8** (150 mg, 0.6 mmol) in toluene (3 cm^3). The mixture was heated under reflux for 20 h and, after evaporation, chromatographed on a silica gel column with chloroform–methanol gradient (from 98:2 to 95:5) to give the *title compound* **9b** (135 mg, 69%). An analytical sample was recrystallized from ethanol, m.p. 132–134 °C (Found: C, 48.2; H, 5.4; N, 21.6. $C_{13}H_{17}N_5O_5$ requires C, 48.3; H, 5.3; N, 21.7%).

7-[(2-*Hydroxyethoxy*)*methyl*]-3-*methylguanine* **9a**.—Compound **9b** (165 mg, 0.51 mmol) was dissolved in 33% aq. diethylamine (2 cm^3) and the solution was heated under reflux for 1 h to form *unblocked product* **9a** in quantitative yield. The mixture was evaporated and the residue was crystallized from methanol. An analytical sample was recrystallized from ethanol, m.p. >300 °C (Found: C, 45.3; H, 5.3; N, 29.3. $C_9H_{13}N_5O_3$ requires C, 45.2; H, 5.5; N, 29.3%).

7-[(2-*Hydroxyethoxy*)*methyl*]-3-*methyl*-1,N-2-(*prop*-1-*ene*-1,2-*diyl*)*guanine* **10a**.—A solution of compound **9a** (90 mg, 0.54 mmol) in DMF (7 cm^3) was stirred with potassium carbonate (148 mg, 1.08 mmol) for 0.5 h at room temperature. Bromoacetone (118 mg, 0.86 mmol) was then added, and the reaction mixture was stirred for 7 h and then evaporated. The residue was dissolved in ethanol and, after evaporation with a portion of silica gel (70–230 mesh), the residue was applied to a silica gel column and chromatographed. Chloroform–methanol (9:1) was used as eluent; fractions containing homogeneous material were evaporated to give a solid, which was recrystallized from ethanol to give fluorescent *title product* **10a** (30 mg, 29%). An analytical sample was crystallized from ethyl acetate–methanol (5:1), m.p. 168–171 °C (Found: C, 52.0; H, 5.4; N, 25.0. $C_{12}H_{15}N_5O_3$ requires C, 52.0; H, 5.5; N, 25.3%).

7-[(2-*Acetoxyethoxy*)*methyl*]-3-*methyl*-1,*N*-2-(*prop*-1-*ene*-1,2-*diyl*)*guanine* **10b**.—A solution of the alcohol **10a** (40 mg, 0.14 mmol) in pyridine (0.7 cm^3) was treated with acetic anhydride (0.25 cm^3, 2.76 mmol) and stirred at room temperature for 1 h. Evaporation and recrystallization from methanol gave the crystalline *product* **10b** (20 mg, 43%), m.p. 114–116 °C (Found: C, 52.1; H, 5.5; N, 21.8. $C_{14}H_{17}N_5O_4$ requires C, 52.7; H, 5.4; N, 21.9%).

Attempted 7→9 *Transglycosylation of Compound* **10b**.—Samples of compound **10b** were heated for 20 min in an oil-bath at 200 and 250 °C. The resulting oil was dissolved in chloroform and analysed by TLC (solvent C). At 200 °C the substrate had remained unchanged, and at 250 °C it had decomposed.

9→7 *Transglycosylation of Compound* **5b**.—A sample of compound **5b** (13 mg, 0.04 mmol) was heated at 200 °C for 10 min. TLC (solvent C) showed the presence of compounds **5b** and **10b** in the reaction mixture. From ^1H NMR analysis [$(CD_3)_2SO$] the ratio of the C-9 and C-7 isomers was determined to be 1:4.

Antiviral Activity Determinations.—Antiviral assays were carried out as described previously.[5,15]

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References

1 U. L. RajBhandary, S. K. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson and K. G. Khorana, *Proc. Natl. Acad. Sci. USA*, 1967, **57**, 751; R. Thiebe and H. Zachau, *Eur. J. Biochem.*, 1968, **5**, 546; H. Kasai, M. Goto, K. Ikeda, M. Zama, Y. Mizuno, S. Takemura, S. Matsuura, T. Sugimoto and T. Goto, *Biochemistry*, 1976, **15**, 898.

2 (*a*) T. Itaya and T. Harada, *J. Chem. Soc., Chem. Commun.*, 1984, 858; (*b*) T. Itaya, K. Matsumoto, T. Watanabe and T. Harada, *Chem. Pharm. Bull.*, 1985, **33**, 2339.

3 J. Boryski and T. Ueda, *Nucleosides, Nucleotides*, 1985, **4**, 477.

4 L.-G. Lin, V. Bakthavachalam, X. M. Cherian and A. W. Czarnik, *J. Org. Chem.*, 1987, **52**, 3113.

5 J. Boryski, B. Golankiewicz and E. De Clercq. *J Med. Chem.*, 1988, **31**, 1351.

6 B. Golankiewicz and W. Folkman, *Nucleic Acids Res.*, 1983, **11**, 5243.

7 J. Boryski, W. Folkman and B. Golankiewicz, *Tetrahedron Lett.*, 1988, **29**, 4163.

8 K. Bazin, X.-X. Zhou, C. Glemarec and J. Chattopadhyaya, *Tetrahedron Lett.*, 1987, **28**, 3275.

9 J. Boryski, T. Ostrowski and B. Golankiewicz, *Nucleosides, Nucleotides*, 1989, **8**, 1271.

10 C. Glemarec, J.-C. Wu, G. Remaud, H. Bazin, M. Oivanen, H. Lönnberg and J. Chattopadhyaya, *Tetrahedron*, 1988, **44**, 1273.

11 J. Kjellberg and N. G. Johansson, *Tetrahedron*, 1986, **42**, 6541.

12 P. Garner and S. Ramakanth, *J. Org. Chem.*, 1988, **53**, 1294.

13 J. Boryski and B. Golankiewicz, *Nucleosides, Nucleotides*, 1989, **8**, 529.

14 B. Golankiewicz, E. Zielonacka-Lis and W. Folkman, *Nucleic Acids Res.*, 1985, **13**, 2443.

15 E. De Clercq, J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence and D. Shugar, *J. Infect. Dis.*, 1980, **141**, 563.

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