Synthesis and Antiviral Activity of Water-Soluble Esters of Acyclovir [9-[(2-Hydroxyethoxy)methyl]guanine]

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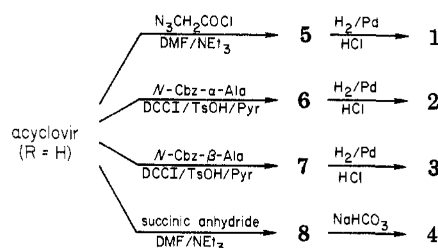
Several water-soluble ester derivatives of acyclovir [9-[(2-hydroxyethoxy)methyl]guanine], i.e., the 2′-*O*-glycyl-, 2′-*O*-α-alanyl-, 2′-*O*-β-alanyl- and 2′-*O*-3-carboxypropionyl esters, were synthesized and evaluated for their antiviral activity in cell culture. The compounds were all prepared directly from acyclovir by application of the usual esterification methods with the appropriate acyl precursors and isolated as their hydrochloride or sodium salts. When assayed in primary rabbit kidney cell cultures against various herpes simplex virus type 1 and type 2 strains, the four acyclovir esters proved almost as active as acyclovir itself, suggesting that they were readily hydrolyzed to release the parent compound.

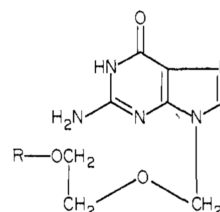
Great progress in the field of antiviral chemotherapy has been made by the advent of acyclovir [9-[(2-hydroxyethoxy)methyl]guanine],[1] BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine],[2] and FIAC [1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine].[3] Unlike the well-established antiherpes compounds, IDU (idoxuridine, 5-iodo-2′-deoxyuridine), TFT [trifluridine, 5-(trifluoromethyl)-2′-deoxyuridine], and *ara*-A (vidarabine, 9-β-D-arabinofuranosyladenine), which exhibit little, if any, selectivity in their antiviral action, acyclovir, BVDU, and FIAC can be considered as highly specific inhibitors of herpes virus replication. They are currently being explored for their clinical potentials in the treatment of herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), and varicella zoster (VZV) infections.

Acyclovir has been the subject of several double-blind placebo-controlled clinical trials. These studies have demonstrated the efficacy of acyclovir in the topical treatment of herpetic keratitis (dendritic corneal ulcers)[4] and the systemic treatment (or prophylaxis) of mucocutaneous HSV-1 infections in immunosuppressed patients (including kidney, bone marrow, and heart transplant recipients[5-7]), primary genital herpes,[8] and acute herpes zoster.[9] For topical use in the treatment of herpetic keratitis, acyclovir has to be applied as a 3% eye ointment; for systemic use in the treatment of HSV and VZV infections, it must be administered intravenously as a bolus infusion of 5 mg/kg (or 250 mg/m^2) every 8 h, thus three times a day, for 5 days or more. Because of its limited solubility in water (~0.2%, 25 °C), acyclovir cannot be given as eye drops or intramuscular injections. Yet, such formulations would seem more practical than the presently used eye ointment and intravenous bolus injections.

We have now endeavored at synthesizing some derivatives of acyclovir, which are much more water soluble than the parent compound and, therefore, applicable for administration as either eye drops or intramuscular injections. These new derivatives are all esters: 9-[[2-(glycyloxy)ethoxy]methyl]guanine hydrochloride (1), 9-[[2-(α-alanyloxy)ethoxy]methyl]guanine hydrochloride (2), 9-[[2-(β-alanyloxy)ethoxy]methyl]guanine hydrochloride (3), and 9-[[2-[(3-carboxypropionyl)oxy]ethoxy]methyl]guanine sodium salt (4) (Scheme I).

Scheme I





1, R = $COCH_2NH_2\cdot HCl$
2, R = $COCH(CH_3)NH_2\cdot HCl$
3, R = $COCH_2CH_2NH_2\cdot HCl$
4, R = $COCH_2CH_2COONa$
5, R = $COCH_2N_3$
6, R = $COCH(CH_3)NHCOOCH_2C_6H_5$
7, R = $COCH_2CH_2NHCOOCH_2C_6H_5$
8, R = $COCH_2CH_2COOH$

Chemistry. The ester derivatives 1–4, were prepared as outlined in Scheme I starting from acyclovir, which was obtained as described previously by Barrio et al.[10] Reaction of acyclovir with azidoacetyl chloride[11] in DMF in the presence of triethylamine gave the azidoacetyl ester 5 in good yield. The corresponding glycyl ester 1, was obtained by reduction of the azido function in 5 by catalytic hydrogenation. In order to avoid hydrolysis of the labile ester function, the hydrogenation was carried out in the presence of an equivalent amount of HCl, and the product was isolated as its crystalline hydrochloride salt from water–ethanol.

The esters 2 and 3 were prepared by reaction of acyclovir with the corresponding *N*-carbobenzoxy-protected amino acid in pyridine, in the presence of *N*,*N*′-dicyclohexylcarbodiimide and a catalytic amount of *p*-toluenesulfonic acid, according to the method of Holmberg et al.[12] The

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Table I. Antiviral Activity of Ester Derivatives of Acyclovir

	minimum inhibitory concentration,[a] μg/mL							
compd	HSV-1 (KOS)	HSV-1 (McIntyre)	HSV-1 (F)	HSV-2 (Lyons)	HSV-2 (G)	HSV-2 (196)	vaccinia virus	vesicular stomatitis virus
1	0.1	0.1	0.1	0.04	0.08	0.07	125	>400
2	0.4	0.4	0.4	0.07	0.2	0.1	70	>400
3	0.2	0.4	0.5	0.1	0.15	0.1	100	>400
4	0.7	0.5	0.7	0.5	0.8	0.4	>400	>400
5	0.1	0.1	0.1	0.04	0.07	0.07	150	ND
acyclovir	0.08	0.1	0.09	0.04	0.06	0.06	80	>400
BVDU	0.01	0.01	0.01	1	0.8	4	4	>400
IDU	0.15	0.2	0.15	0.2	0.2	0.4	0.1	>400

[a] Required to reduce virus-induced cytopathogenicity in primary rabbit kidney cell cultures by 50%. Average values for two or three separate determinations. Abbreviations: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; BVDU, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine; IDU, 5-iodo-2'-deoxyuridine; ND, not determined.

thus obtained N-protected 2-*O*-alanyl derivatives **6** and **7** were purified by column chromatography on silica gel and subsequently subjected to catalytic hydrogenation in the presence of 1 equiv of HCl to give, after crystallization from H_2O–EtOH, the α- and β-alanyl esters as their hydrochloride salts **2** and **3**, respectively.

For the synthesis of the sodium salt of the 3-carboxypropionyl ester **4**, acyclovir was reacted with succinic anhydride in DMF in the presence of triethylamine to give the free carboxy derivative **8** in excellent yield. After conversion to the sodium salt with $NaHCO_3$ and purification by column chromatography on XAD-2, **4** was obtained in an overall yield of 76%.

Biological Results and Discussion

The acyclovir esters (**1–5**) were as active or only slightly less active than acyclovir itself in inhibiting the cytopathogenicity of HSV-1 and HSV-2 in primary rabbit kidney cells (Table I). Like the parent compound, the esters **1–5** showed little activity against vaccinia virus and no activity at all against vesicular stomatitis virus. Cytotoxicity, as reflected by a microscopically detectable alteration of normal cell morphology, was not observed with any of the compounds at the highest concentration tested (400 μg/mL). The antiviral data obtained for the acyclovir esters suggest that they are readily hydrolyzed to release the parent compound.

This was confirmed by the results of a separate experiment, where the rate of ester hydrolysis was followed by monitoring by HPLC the amount of acyclovir released (see Experimental Section). Both the glycine (**1**) and the β-alanine (**3**) esters of acyclovir were completely hydrolyzed within 1 day (up to 50% after 4 h) when incubated at 37 °C in cell culture medium (Eagle's minimal essential medium, pH 7.4, supplemented with 3% heat-inactivated calf serum). Thus, even in the absence of any esterase activity, total hydrolysis of the acyclovir esters occurred within the time limits of the antiviral assays (3–4 days).[13] Comparable results were obtained for the hydrolysis of the esters at 37 °C in 0.15 M phosphate buffer at pH 7.4.

One of the esters, viz., **1**, was further evaluated for its antiviral efficacy in a representative animal model infection, namely, HSV-1 keratitis in rabbits. When administered as eye drops at a concentration of 1% (at which acyclovir itself could not be dissolved) in isotonic borate buffer (pH 5.7), **1** effectively suppressed the development of both epithelial and stromal keratitis, and iritis therewith associated.[14] In contrast to their behavior at pH 7.4, the acyclovir esters remained stable when stored for 6 days at room temperature in the isotonic borate buffer (pH 5.7), as determined by TLC (EtOH–HOAc, 8:2).

Acyclovir esters, such as those described here, should be more practical for clinical use than their parent compound. For topical treatment of herpetic keratitis they can be applied as eye drops at 1% or even higher concentrations, if necessary. In fact, the solubility of the esters **1–4**, as determined in an isotonic phosphate or borate buffer at pH 7.4, amounted to about 6%. Because of its low solubility in water (0.2%), acyclovir cannot be applied in eye drops at therapeutically effective concentrations. This explains why it is generally prescribed as an ointment at 3%. While the acyclovir esters seem ideally suited for topical use as eye drops, they could also be pursued in the systemic treatment of those herpes virus infections (mucocutaneous herpes simplex, primary genital herpes, and acute herpes zoster) that respond well to intravenous acyclovir treatment.[5-9] In contrast with acyclovir which has to be administered as repeated intravenous bolus infusions of rather large volumes, the acyclovir esters could be given in much smaller volumes and may, therefore, be amenable to intramuscular injections.

The practical advantage of the water-soluble esters of acyclovir is that they permit the administration of large quantities of the drug for either topical use (i.e., eye drops) or parenteral administration. On the basis of the results of the hydrolysis experiment at pH 7.4, we may expect that upon adminsitration, the acyclovir esters are readily hydrolyzed to release the parent compound, so that the ultimate activity, possible toxicity and other biological effects, would be attributed to the parent compound. However, further experiments about the stability of the acyclovir esters, i.e., when submitted to sterilization procedures, are needed to establish the practical usefulness of these esters as candidate prodrugs of acyclovir.

Experimental Section

Melting points were determined with a Büchi-Tottoli apparatus and were uncorrected. IR spectra were run on a Model 257 Perkin-Elmer infrared spectrophotometer. UV spectra were recorded on a Beckman Model 2 spectrophotometer. Mass spectra were recorded on a AEI MS-12 apparatus. NMR spectra were recorded on a 60-MHz Hitachi Perkin-Elmer R-24 or on a Varian XL-100 apparatus with tetramethylsilane as internal standard (signals are described as: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br s, broad signal). Microanalyses were performed by Janssen Pharmaceutica N.V. (Beerse, Belgium).

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The purity of all the compounds was checked, and their R_f values were determined on "Fertigplatten" Riedel-de-Haën Kieselgel 60 F254, and the spots were located by UV light (254 nm) or by iodine vapors.

9-[[2-(Azidoacetoxy)ethoxy]methyl]guanine (5). A solution of 0.84 mL (8.4 mmol) of azidoacetyl chloride in 5 mL of dry DMF was added dropwise over a period of 10 min to an ice-cooled, stirred suspension of 1.25 g (5.55 mmol) of acyclovir[10] and 0.78 mL (5.55 mmol) of triethylamine in 40 mL of dry DMF. After the mixture was stirred for 45 min at 0 °C, additional azidoacetyl chloride (0.11 mL, 1.1 mmol) in 1 mL of DMF was added, and stirring was continued for 15 min. The reaction was quenched by the addition of 15 mL of 7% $NaHCO_3$, and the mixture was evaporated in vacuo. The residue was triturated with 20 mL of a mixture of CH_2Cl_2–Et_2O–EtOH (5:5:1), and the resulting solid was collected by filtration, washed thoroughly with cold water, and recrystallized from hot water, after treatment with active charcoal, to yield 1.02 g (62%) of **5**: mp 175–177 °C dec; MS, *m/z* 308 (M^+), 151 (base + H); IR (KBr) 2110 (N_3), 1745 (COOR) cm^{-1}; UV (phosphate buffer, pH 7) λ_{max} 253 nm (ϵ 8.42 × 10^3), 270 (sh); TLC R_f 0.27 ($CHCl_3$–MeOH, 8:2), R_f 0.60 (EtOH–HOAc, 8:2).

9-[[2-(Glycyloxy)ethoxy]methyl]guanine Hydrochloride (1). A mixture of 1.23 g (4 mmol) of **5**, 1.0 g of 10% Pd/C, and 4 mL of 1.0 N HCl in H_2O–EtOH (1:1, 250 mL) was hydrogenated at a H_2 pressure of 40 psi for 1.5 h. The catalyst was removed by filtration and washed several times with water. The filtrate and washings were evaporated in vacuo, and the residue was crystallized twice from H_2O–EtOH, yielding 0.83 g (65%) of **1**: mp 148–150 °C; IR (KBr) 3200–2350 (NH_3^+), 1750 (COOR) cm^{-1}; UV (0.1 N HCl) λ_{max} 253 nm (ϵ 8.53 × 10^3), 275 (sh); NMR (60 MHz, Me_2SO-d_6/D_2O) δ 3.85 (s, 2 H, NCH_2CO), 4.25 (m, 4 H, CH_2CH_2), 5.5 (s, 2 H, OCH_2N), 8.3 (s, 1 H, 8-H); TLC R_f 0.29 (EtOH–HOAc, 8:2). Anal. ($C_{10}H_{14}N_6O_4$·HCl) C, H, N.

9-[[2-(α-Alanyloxy)ethoxy]methyl]guanine Hydrochloride (2). A mixture of 0.99 g (4 mmol) of acyclovir, 1.026 g (4.3 mmol) of *N*-carbobenzoxy-DL-α-alanine (Aldrich Chemical Co.), 0.04 g of anhydrous *p*-TsOH, and 1.755 g (5.6 mmol) of *N,N'*-dicyclohexylcarbodiimide in 80 mL of dry pyridine was stirred at room temperature for 1 day. Acetic acid (1 mL) was added, and the mixture was stirred for 1 h more. The reaction mixture was filtered, and the precipitate was washed thoroughly with hot MeOH. The filtrate and washings were evaporated, and the residue was applied to a column of silica gel (50 g) and eluting with $CHCl_3$–MeOH (9:1). Finally, crystallization from MeOH gave 1.15 g (67%) of the protected α-alanyl ester **6**: mp 145–147 °C; UV (MeOH) λ_{max} 255 nm (ϵ 9.87 × 10^3), 270 (sh); TLC R_f 0.40 ($CHCl_3$–MeOH, 8:2). A mixture of this derivative **6** (0.662 g, 1.54 mmol) and 3.1 mL of 0.5 N HCl in H_2O–MeOH (1:1, 200 mL) was hydrogenated in the presence of 0.30 g of 10% palladium on charcoal at a H_2 pressure of 40 psi for 2 h. The catalyst was filtered off and washed with water, and the filtrate and washings were evaporated in vacuo. The solid residue was crystallized from H_2O–EtOH, yielding 0.354 g (71%) of **2**: mp 153–155 °C; UV (0.1 N HCl) λ_{max} 254 nm (ϵ 1.10 × 10^4), 273 (sh); NMR (100 MHz, Me_2SO-d_6) δ 1.36 (d, 3 H, CH_3, J = 8 Hz), 3.72 (m, 2 H, $COOCH_2CH_2O$), 4.04 (q, 1 H, CH, J = 8 Hz), 4.26 (m, 2 H, $COOCH_2$), 5.38 (s, 2 H, OCH_2N), 6.74 (s, 2 H, 2-NH_2), 7.84 (s, 1 H, 8-H), 8.56 (br s, 3 H, NH_3^+), 10.9 (br s, 1 H, 1-NH); TLC R_f 0.23 (EtOH–HOAc, 8:2). Anal. ($C_{11}H_{16}N_6O_4$·HCl) C, H, N.

9-[[2-(β-Alanyloxy)ethoxy]methyl]guanine Hydrochloride (3). The β-alanyl ester **3** was prepared in a manner identical with **2**, with 0.99 g (4 mmol) of acyclovir and 1.026 g (4.3 mmol) of *N*-carbobenzoxy-β-alanine (Aldrich Chemical Co.) to afford 1.24 g (72%) of **7** as a crystalline solid from MeOH: mp 147–148 °C; UV (MeOH) λ_{max} 254 nm (ϵ 9.91 × 10^3), 270 (sh); NMR (60 MHz, pyridine-d_5) δ 2.65 (t, 2 H, CH_2COO, J = 6 Hz), 3.65 (m, 4 H, NCH_2CH_2 and $COOCH_2CH_2O$), 4.20 (m, 2 H, $COOCH_2$), 5.20 (s, 2 H, OCH_2N), 5.50 (s, 2 H, $PhCH_2O$), 7.3 (m, 5 H, phenyl), 8.1 (s, 1 H, 8-H); TLC R_f 0.41 ($CHCl_3$–MeOH, 8:2). Hydrogenation of **7** (0.99 g, 2.3 mmol) in the presence of an equivalent amount of HCl gave, after workup of the reaction mixture as was described for **2**, 0.590 g (77%) of **3**: mp 202–204 °C dec; UV (0.1 N HCl) λ_{max} 254 nm (ϵ 1.09 × 10^4), 273 (sh); NMR (100 MHz, Me_2SO-d_6) δ 2.69 (m, 2 H, CH_2COO), 3.01 (m, 2 H, NCH_2CH_2), 3.71 (m, 2 H, $COOCH_2CH_2O$), 4.15 (m, 2 H, $COOCH_2$), 5.38 (s, 2 H, OCH_2N), 6.71 (s, 2 H, 2-NH_2), 7.84 (s, 1 H, 8-H), 8.08 (br s, 3 H, NH_3^+), 10.86 (br s, 1 H, 1-NH); TLC R_f 0.23 (EtOH–HOAc, 8:2). Anal. ($C_{11}H_{16}N_6O_4$·HCl) C, H, N.

9-[[2-[(3-Carboxypropionyl)oxy]ethoxy]methyl]guanine Sodium Salt (4). A solution of 1.125 g (5 mmol) of acyclovir, 1.0 g (10 mmol) of succinic anhydride, and 0.71 mL of triethylamine in 75 mL of dry DMF was heated at 60 °C in an oil bath for 21 h. After the solution was cooled, the volatile constituents were evaporated in vacuo, and the residue was taken up in 40 mL of ice–water and acidified to pH 2 with 2 N HCl. The white precipitate was collected by filtration, thoroughly washed with ice–water, and dried in vacuo over P_2O_5 at 40 °C to yield 1.41 g (87%) of **8**. An analytical sample was obtained by crystallization from MeOH: mp 195–196 °C; MS, *m/z* 175 (M^+ – base), 151 (base + H), 101 (3-carboxypropionyl); IR (KBr) 3000 (OH), 1740 (COOR), 1695 (COOH) cm^{-1}; TLC R_f 0.58 (EtOH–HOAc, 8:2).

To the above-prepared 3-carboxypropionyl ester **8** (0.325 g, 1 mmol) was added 20 mL of a 1.25% $NaHCO_3$ solution, and the mixture was stirred for 10 min at room temperature. The almost clear solution was filtered, and the filtrate was concentrated and applied on the top of an XAD-2 column (20 g, 100–200 μm, 2-cm diameter). The column was eluted with H_2O, and the UV-absorbing fractions were collected, concentrated in vacuo to a volume of 30 mL, and finally lyophilized, yielding 0.31 g (88%) of pure **4**: mp 187–189 °C; IR (KBr) 1735 (COOR), 1575 and 1400 (COO^-) cm^{-1}; UV (0.1 N HCl) λ_{max} 252 nm (ϵ 1.02 × 10^4), 273 (sh); NMR (60 MHz, Me_2SO-d_6/D_2O) δ 2.3 (t, 4 H, $OCCH_2CH_2CO$), 3.9 (m, 4 H, $COOCH_2CH_2O$), 5.3 (s, 2 H, OCH_2N), 7.75 (s, 1 H, 8-H); TLC R_f 0.58 (EtOH–HOAc, 8:2).

Stability of the Ester Derivatives 1 and 3 in Cell Culture Medium. A solution of 10 or 100 μg/mL of the ester derivatives in cell culture medium [Eagle's minimal essential medium supplemented with 3% heat (56 °C, 30 min) inactivated calf serum] was incubated at 37 °C. The rate of hydrolysis was monitored by HPLC analysis, with 2'-deoxyinosine as internal standard. A 250 mm × 4.6 mm i.d. column packed with 10 μm ODS silica was used (Lichrosorb RP-18, 10 μm). The mobile phase was water containing 10% acetonitrile and 0.5% HOAc.

Biological Activity. The origin of the herpes simplex virus strains (HSV-1 (KOS, McIntyre and F) and HSV-2 (Lyons, G and 196)) and the technique used to measure inhibition of virus-induced cytopathogenicity have been described previously.[13]

Acknowledgment. This investigation was supported by Grant 3.0048.75 from the Belgian "Fonds voor Geneeskundig Wetenschappelijk Onderzoek", Grant 76/81-IV from the Belgian "Geconcerteerde Onderzoeksacties", and Grant OT/VII/37 from the "Onderzoeksfonds (Katholieke Universiteit Leuven)". The excellent technical assistance of Anita Van Lierde is gratefully acknowledged.

Registry No. 1, 84499-61-6; 1 free base, 84499-62-7; 2, 84499-63-8; 2 free base, 84499-64-9; 3, 84499-65-0; 3 free base, 84499-66-1; 4, 84499-67-2; 5, 84499-68-3; 6, 84499-69-4; 7, 84499-70-7; 8, 59298-42-9; azidoacetyl chloride, 30426-58-5; acyclovir, 59277-89-3; *N*-carbobenzoxy-α-alanine, 1142-20-7; *N*-carbobenzoxy-β-alanine, 2304-94-1; succinic anhydride, 108-30-5.