

## SYNTHESIS OF ACYCLOVIR AS AN ANTIHERPES-VIRUS DRUG

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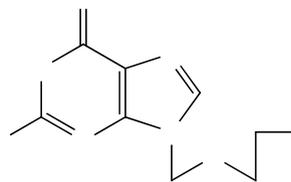
### ABSTRACT

A one-pot process for high-yield regioselective synthesis of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir), an antiherpetic agent, was achieved from guanine via the steps of reacting of  $N^2, N^9$ -diprotected guanine with 1-acetyl-2-acetylmethoxy-ethyleneglycol in presence of phosphoric acid or polyphosphoric acid. Total yield of product was 59%. The obtained acyclovir meets the standards in the British Pharmacopoeia 2007 (BP2007). Its activity as inhibitor of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) was evaluated according to the plaque reduction assay method and compared with the standard compound. The synthesized acyclovir showed a similar activity as the control.

**Keywords:** Acyclovir, Herpes simplex virus, Nucleoside, One-pot synthesis.

### I - INTRODUCTION

Virus was a cause of many dangerous diseases and variety of epidemic diseases in large area. It is important that antibiotics have no or almost no effect on viruses. Although the production of herpes vaccine is desired by many countries but it still stays on a testing level. The prevention of herpes infection is a special problem because the disease often recurred in present of antibody. Therefore, development of anti-virus drugs is important, necessary and still be a leading interest.



**1:** *Acyclovir*

Acyclovir, {9-[(2-hydroxyethoxy)methyl]guanine} (ACV **1**), was first reported by

Schaeffer and co-workers [1] and is a nucleoside analogue. It acts selectively on herpes-infected cells. It has specific inhibition effects on replication of herpes virus simplex type 1 and type 2 (HSV1 and HSV2) and *Varicella zoster* virus that cause almost no intoxication to the host cells. From nearly three decades ago, ACV was used as a primary drug to treat herpes virus, especially genital herpes and herpes simplex encephalitis and herpes in immunity deficient people [2].

ACV has no activity against viral pathogens until it is converted to an active form acyclovir triphosphate. Its mechanism of action is initiated by the viral enzyme *Thymidine kinase*; subsequently, human *Cellular kinase* perform the second and the third phosphorylation steps to complete the process (figure 1). Acyclovir triphosphate, the active form of acyclovir, is present in 40- to 100-fold higher concentrations in herpes simplex virus (HSV)-infected cells than in uninfected cells. As a result, the selectivity of ACV is significant [3, 4].

Acyclovir triphosphate has a two-pronged mechanism of action: (1) it competes with 2-deoxyguanosine triphosphate (dGTP) as a substrate for viral DNA polymerase and (2) once it becomes incorporated into the replicating viral DNA, it acts as a chain terminator because it does not have a terminal 3-hydroxyl group [3].

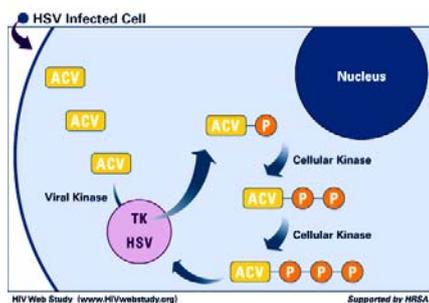


Figure 1: Acyclovir mechanism of action

ACV was prepared in different forms such as tablets (200 mg, 400 mg, 800 mg), capsules 200 mg, powder for injection, oral suspension, creams for skin 5% and for eyes 3%. After oral administration, ACV is absorbed slowly and not completely, bioavailability of only 10-20% [9].

ACV rapidly attracted attention of chemist, as well as pharmacist by virtue of its selective anti-virus activity and effect. There were many efforts for synthesis of ACV by different ways reported. Among divert orientations of synthesizing ACV, it could be accounted 5 common and most effective pathways. The most common pathway departed from guanine with effectiveness and simplicity where guanine was acylated [5]. The important problem is to decrease the N<sup>7</sup> substituted isomer and selectively acylating into N<sup>9</sup> position. Our synthesis of ACV is showed in figure 2.

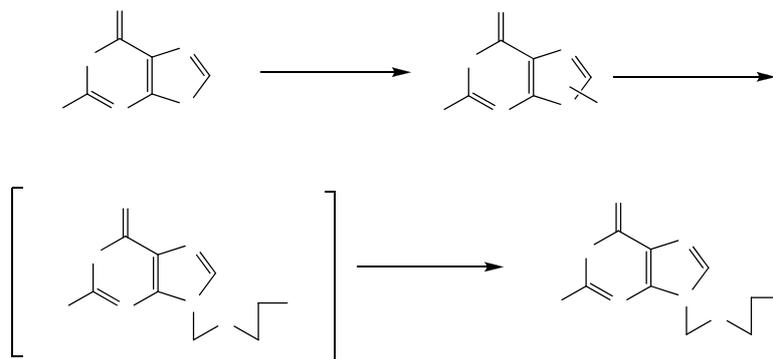


Figure 2: Pathway for synthesis of acyclovir

## II - EXPERIMENTAL

### Preparation of dioxolane-diacetate (1-acetyl-2-acetylmethoxy-ethyleneglycol)

Dioxolane **5** (224 g, 99%, 211.6 ml, 3 mol) was added drop wise to a solution of p-toluenesulfonic acid monohydrate (10 g, 0.053 mol) in acetic anhydride (306 g, 283.3 ml, 3 mol) and acetic acid (45 g, 42.9 ml, 0.75 mol) precooled to below 10°C, at the rate that maintained the reaction temperature under 30°C.

The solution was then stirred for an additional hour at a temperature under 30°C, and then distilled. The first portion of distillate was collected at a temperature of 40 - 80°C/1.5 mmHg and contained acetic acid, acetic anhydride, and other materials. The second portion of distillate was collected at 80 - 95°C. at 0.75 mmHg and redistilled to give pure **7** in a yield of 431.5 g (81.7%). <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz, δ ppm): 2.06, 2.08 (s, 6H, 2xCH<sub>3</sub>), 3.85, 4.21 (s, 4H, OCH<sub>2</sub>CH<sub>2</sub>O), 5.28 (s, 2H, OCH<sub>2</sub>O).

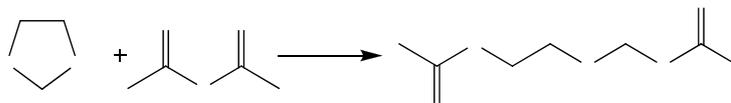


Figure 3: Preparation of Dioxolane-diacetate

### Preparation of acyclovir

A mixture of guanine (**3**, 32.13 g, 213 mmol), acetic anhydride (320 ml) and acetic acid (480 ml) was refluxed overnight, during which time the mixture became almost clear. The liquid was removed by evaporation in reduced pressure. To the residue was added toluene (300 ml), **7** (75 g, 426 mmol), and phosphoric acid (85.5%, 1.5 ml) with stirring. The resulting mixture was refluxed for 6 - 7 hours with vigorous stirring.

Toluene and acetic anhydride were then removed under reduced pressure and the residue was heated at 80°C (oil bath) under reduced pressure (20 mm Hg) for another 2 hours, cooled to room temperature, triturated with ethyl acetate (100 ml), and stirred at room temperature overnight.

The resulting solid was collected by filtration, washed twice with ethyl acetate (25 ml), dissolved in ammonium hydroxide (30%, 300 ml). The solution became clear and solution was stirred at room temperature overnight. The mixture was concentrated to dryness under reduced pressure. The residue was treated with methanol (150 ml) and the resulting mixture was heated at 80°C for 1 hour, cooled, and allowed to stand at room temperature overnight. The resulting solid was collected by filtration and recrystallized from water (1700 ml) using 1 gram of activated carbon. The filtrate was cooled to room temperature, and stored in the refrigerator overnight. The resulting solid was collected by filtration, washed twice with methanol (20 ml), and dried to give pure acyclovir **1** (30 g, 63%). mp. 242 - 244°C (H<sub>2</sub>O). UV λ<sub>max</sub> (MeOH-H<sub>2</sub>O; 1-1) nm: 251, 276 (sh); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>, 500 MHz, δ ppm): δ 3.34, 3.46 (s, 4H, CH<sub>2</sub>CH<sub>2</sub>), 4.65 (br, 1H, OH), 5.34 (s, 2H, OCH<sub>2</sub>O), 6.48 (s 2H, NH<sub>2</sub>), 7.80 (s, 1H,

H-8), 10.62 (s, 1H, NH). EI-MS m/e (%) 226 [M<sup>+</sup>] (100), 152 [M<sup>+</sup>-C<sub>3</sub>H<sub>6</sub>O<sub>2</sub>] (42). The mother liquor was concentrated to give a second crop (2.6 g) which contained the N<sup>7</sup>-isomer. C

### Antitherpetic activity evaluation

#### a) Cells and viruses

Vero cells (Green Africa Monkey Kidney) were propagated in culture medium (Medium Essential **5** Medium (MEM, **6** GIBCO), supplemented with 3% fetal bovine serum (FBS, Sigma), glutamine, non-essential amino acid, and antibiotics).

The 5 clinical HSV isolates used in this study included 2 HSV-1 and 3 HSV-2.

#### b) Method of plaque reduction assay (PRA)

Confluent Vero cell monolayer in 24-well culture plates were infected with 200 μl of 10 fold dilutions from 10<sup>-5</sup> to 10<sup>-2</sup> of virus stocks per well. After 1 hour incubation at 37°C x 5%CO<sub>2</sub> with gently rocking, inoculates were discarded and cells were incubated with culture medium and 1.0% methylcellulose (Sigma) with the presence of increasing antiviral concentrations of 0, 1, 5, 15, and 50 μM. Plaques were counted at 72 h post inoculation after cells were treated with formaldehyde and stained with crystal violet. IC<sub>50</sub>s were determined on log papers and defined as the minimum concentration of ACV that reduced the numbers of plaque by 50% compared to the cell controls (absence of ACV). IC<sub>50</sub>s ≥ 7 μM were considered resistant to ACV.

## III - RESULTS AND DISCUSSION

### 1. Preparation of acyclovir

A method for the preparation of ACV is

provided that is suitable for the commercial manufacture of the product. In the first step, guanine is acetylated using acetic anhydride, acetic acid, and phosphoric acid. In the second step, diacetyl guanine is alkylated at the N<sup>9</sup>-position using CH<sub>3</sub>COOCH<sub>2</sub>O(CH<sub>2</sub>)<sub>2</sub>OCOCH<sub>3</sub>, acetic anhydride and phosphoric acid or polyphosphoric acid. The acetyl groups are then removed as desired.

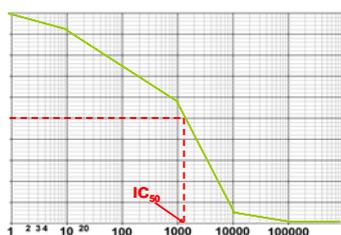


Figure 4: Plot for determination of IC<sub>50</sub>

## 2. Acetylation of guanine

In the first step of the reaction, the 2-amino group of guanine is protected to prevent it from being alkylated in the second step of the reaction. The choice of protecting group may effect the ultimate yield of product, in that protecting groups are removed with varying degrees of difficulty. In general, in the process of protecting the 2-amino group, the N<sup>9</sup> group usually also reacts with the protecting group. Acylation, and in particular, acetylation, activates the N<sup>9</sup>-position toward alkylation in the second step, and therefore, is desirable.

Diacetylguanine, which has been used as an intermediate in the production of acyclovir, has been prepared using several methods. Guanine has been acetylated using acetic anhydride in N,N-dimethylacetamide to give diacetylguanine in 90.5% yield [6]. This reaction produces a product which is grey in color due to the high reaction temperature used (160°C for 7 hours). In general, it is preferred to use a symmetrical anhydride in the reaction scheme that corresponds to the acid used. For example, acetic acid is preferably used in combination with acetic anhydride, and propionic acid is preferably used in combination with propionic anhydride.

Guanine has also been acetylated in acetic anhydride and acetic acid to give different products depending on the work-up conditions [7]. For example, after the reaction mixture becomes an almost clear solution, if solvents are removed by distillation, only diacetyl guanine is obtained in 95% yield. However, the addition of water at 60°C. followed by stirring at room temperature overnight produces N<sup>2</sup>-acetylguanine in 94.4% yield. If the reaction mixture is merely cooled down, a mixture of mono- and di-acetylguanine is produced.

## 3. Preparation of dioxolane-diacetate (7)

The dioxolane ring is opened using acetic anhydride as the ring-opening reagent in the presence of catalytic amounts of p-toluenesulfonic acid. This provided dioxolane-diacetate in 80% yield after distillation by the method of Chen et al. [8].

## 4. Alkylation of diacetylguanine

In this step of the synthesis, the diacetylated guanine from previous step is alkylated in the N<sup>9</sup>-position to produce 2-acetamido-9-(2-acetyloxyethyl)methyl)guanine.

Matsumoto, et al., have studied the effect of solvent, acid catalyst and reaction temperature on the yield of the alkylation of diacetylguanine with 2-oxo-1,4-butanediol diacetate ("dioxolane diacetate") to produce N<sup>2</sup>,O-diacetylacyclovir [7]. Among the acid catalysts tested (p-toluenesulfonic acid, sulfanilic acid, p-nitrobenzenesulfonic acid, 2,4-dinitrobenzene sulfonic acid, iron(II) sulfate, and zinc chloride), Matsumoto reported that p-toluenesulfonic acid and sulfanilic acid exhibited the highest catalytic activity. In a preferred reaction scheme, a combination of an acid and anhydride are used.

## 5. Deacetylation of acyclovir diacetate

Both methylamine and ammonium hydroxide proved to be good deacetylating agents. Although the working-up is easier using methylamine than it is using ammonium hydroxide, on an industrial scale, the smell of methylamine may dictate the preferred use of ammonium hydroxide as a deacetylation agent.

The synthesized ACV has been analyzed in the National Institute for Drug Quality Control. It meets all the standards of BP 2007.

## 6. Biological activity

ACV was evaluated against HSV-1 and HSV-2. The effects of standard ACV and tested ACV on 5 clinical HSV isolates included HSV-1 and HSV-2 were tested in parallel with their antiviral activity. The results are given in table 1.

Table 1: The antiviral activity of ACV compared to the standard ACV

Order	Clinical HSV isolate	Type	IC <sub>50</sub>				Correlated coefficient
			Standard		Tested		
1	06003	HSV-2	1.70	S	1.20	S	0.95
2	07069	HSV-1	0.53	S	0.57	S	
3	07089	HSV-2	0.91	S	0.10	S	
4	07092	HSV-2	2.80	S	2.60	S	
5	07094	HSV-1	0.89	S	0.53	S	

S: Sensitive.

The tested ACV showed the anti-HSV activity at low concentration and very corresponds with standard ACV. The correlated factor is 0.95.

activity of the synthesized ACV was show to be comparable (95%) to the standard ACV. This synthesized ACV was approved by National Institute of Drug Quality Control to meet the quality standard of BP 2007.

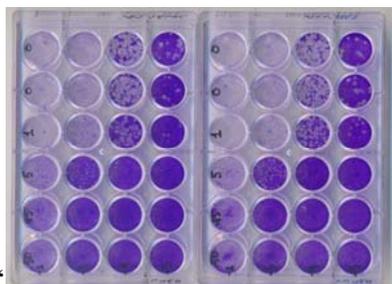


Figure 5: Antiherpetic activity of standard (left) and tested (right) acyclovir

## IV - CONCLUSION

This report describes a one-pot process for the preparation of acyclovir from guanine. Reacting N<sup>2</sup>,N<sup>9</sup>-diprotected guanine with 1-acetyl-2-acetylmethoxy-ethyleneglycol in present of phosphoric acid as catalyst provide ACV with high yield and good regioselectivity. Therefore, the purification was simple, inexpensive and conforming to pilot scale. The chemicals and the solvents were common and friendly with environment. The antiherpetic

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