

A Synthetic Study on Cyclic Phosphate Derivatives of Seconucleosides as Potential Antiviral Agents (II): Synthesis of 3',5'-Cyclic Phosphates of 6-aza-2'-azido-secouridine

Dae Hong Shin, Jae-Wook Yang, Joong Hyup Kim*, Chong-Kyo Lee**

Won-Keun Chung and Moon Woo Chun

College of Pharmacy, Seoul National University, Seoul 151-742, Korea

*Korea Institute of Science and Technology, Seoul 131, Korea

**Korea Research Institute of Chemical Technology

(Received October 12, 1992)

Abstract □ The synthesis of 6-aza-3',5'-*p*-nitrophenylphosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secouridine and 6-aza-3',5'-phosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secouridine ammonium salt are described and they were evaluated for antiviral activity primarily against DNA and RNA viruses and found to be inactive, but no significant cytotoxic effect was observed against Vero and HeLa cell.

Keywords □ Acyclonucleosides, cyclic phosphate of 2',3'-seconucleoside, 6-azauridine.

In previous report¹⁾, we described the syntheses of several 3',5'-cyclic phosphates of secouridine²⁾ and securibavirin³⁾ (Fig. 1).

We report herein the synthesis of 6-aza-3',5'-*p*-nitrophenylphosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secouridine **2** and 6-aza-3',5'-phosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secouridine ammonium salt **1**.

The synthesis of 6-aza-2'-azido-2'-deoxy-2',3'-secouridine was initiated by oxidation of sugar moiety of 6-azauridine **3** with sodium periodate⁴⁾. After reduction of the above dialdehyde **4** with sodium borohydride, we protected the 3',5'-OH group as isopropylidene group by using acetone and DMP under anhydrous acidic condition. The remaining 2'-OH group was tosylated in anhydrous pyridine for substitution reaction.

The azidation of the tosylate **7** with sodium azide gave protected 2'-azido compound **8**. And then, the protected 2'-azido compound **8** was deprotected with 80%-acetic acid to give 6-aza-2'-azido-2'-deoxy-2',3'-secouridine **9**.

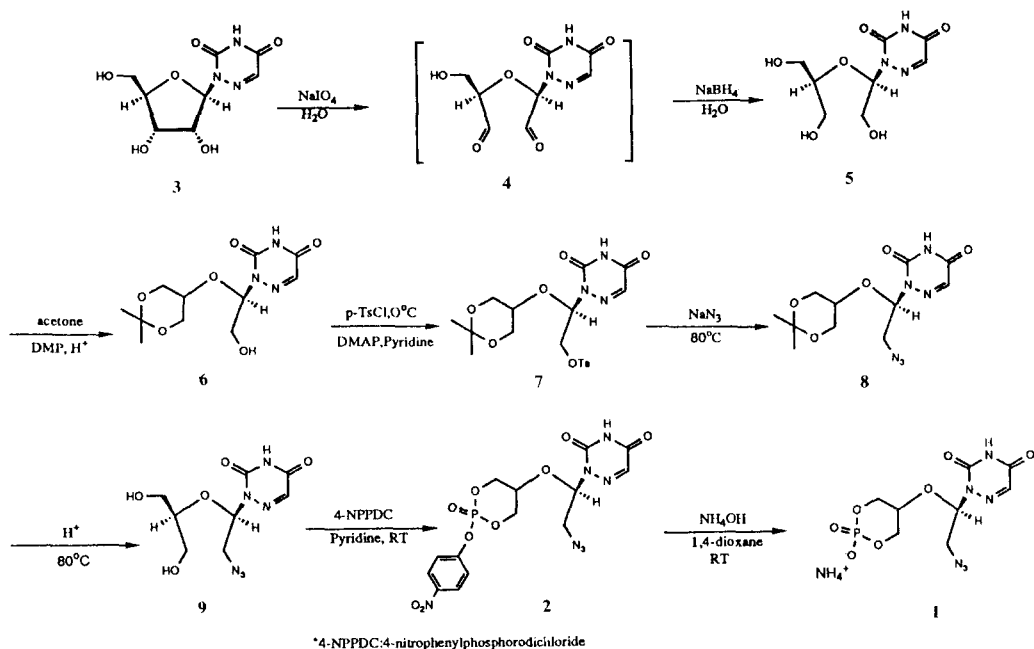
The 2'-azido secoderivative **9** was treated with 4-nitrophenyl phosphorodichloridate⁵⁾ in anhydrous

acetonitrile in the presence of pyridine at room temperature to yield their respective 3',5'-cyclic-*p*-nitrophenyl phosphate **2** as diastereomeric mixtures.

When we treated each diastereomer with 28% NH₄OH after separation, identical products **1** were obtained from each diastereomer. However, we couldn't separate each diastereomer because it was too difficult to separate them. But there was difference in the rate of ammonolysis of two diastereomer. The more polar diastereomer formed ammonium salt more rapidly than less polar diastereomer. This difference seems to be due to the preferable structure of the more polar diastereomer for removing of *p*-nitrophenyl group.

EXPERIMENTAL

Proton NMR spectra were measured at 80 MHz on a Bruker instrument and chemical shifts were reported in δ units relative to internal tetramethylsilane. Infrared spectra were measured on Perkin-Elmer 735B and Analect FX-6160 FT-IR and frequencies are given in reciprocal centimeters.



Scheme 1.

The extent of reaction was checked on thin layer chromatography. Analytical thin layer chromatography was performed on precoated silica gel (0.25 mm, 60G254, Merck) and was used silica gel (Kiesel gel, 70-230 mesh, Merck) for column chromatography and all the chromatographic solvents were distilled before used.

6-Aza-2',3'-secouridine, 5

To the solution of the 6-Aza-2',3'-secouridine (1.54g, 6.29 mmol) **3** in 25 ml of water was added sodium periodate (1.48g, 6.92 mmol) at room temperature. After the reaction mixture stirred for 4 hrs, 30 ml of absolute ethyl alcohol was poured into the reaction mixture in order to remove inorganic salt. The reaction mixture was stirred for 20 min, then the precipitates were filtered. The filtrate was evaporated to dryness under diminished pressure.

To the dialdehyde in 4 ml of water was added sodium borohydride (238 mg, 6.29 mmol). After stirred for 1 hr, at room temperature, the reaction mixture was neutralized with Dowex 50W[H⁺] resin. The resin was removed by filtration, and the solvents were evaporated. The reaction mixture was purified with column chromatography (CHCl_3 : CH_3

OH =5:1, silica gel=20g). The product was obtained as a white foam **5** (1.1g, 80%).

¹H-NMR (DMSO): 9.94 (s, 1H, H-3), 7.47 (s, 1H, H-5), 5.91-5.75 (t, 1H, H-1'), 5.48 (m, 3H, OH-2',3',5'), 3.68-3.12 (m, 7H, H-2',3',4',5').

IR (neat): 3400 (OH, Broad), 1800 (pyrimidinone).

6-Aza-3',5'-O-Isopropylidene-2',3'-secouridine, 6

Compound **5** (455 mg, 1.84 mmol) and $p\text{-TsOH}$ (158 mg, 0.92 mmol) were dried for 20 min, in the flask was exchanged to nitrogen gas, then DMF (10 ml) was added under anhydrous condition. To the solution was added 2,2-dimethoxypropane (1 ml) with a dropwise injection. The reaction mixture was stirred at room temperature for 24 hr. The reaction mixture was quenched with saturated NaHCO_3 solution (qs).

After the reaction mixture was filtered, the residue was evaporated *in vacuo*, and purified with column chromatography (CHCl_3 : CH_3OH =15:1, silica gel =25g). The product was obtained as a white solid **6** (318 mg, 69%).

¹H-NMR (CDCl_3): 9.85 (s, 1H, H-3), 7.47 (s, 1H, H-5), 5.92 (t, 1H, H-1'), 4.42-3.38 (m, 8H, H-2',3',4',5' and OH-2'), 1.37 (s, 6H, acetoneid).

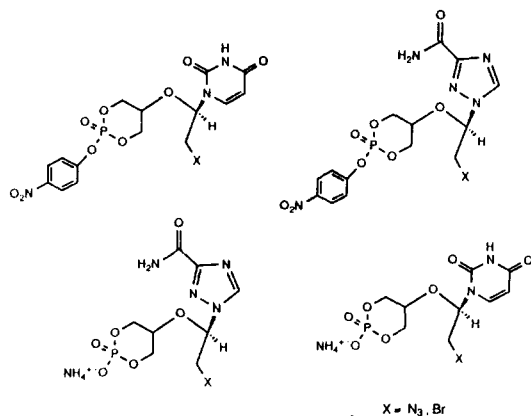


Fig. 1.

IR (neat): 3350 (-OH), 1680 (C=O).

6-Aza-3',5'-O-Isopropylidene-2'-O-tosyl-2',3'-secouridine, 7

Compound **6** (252.3 mg, 0.87 mmol), p-TsCl (334.2 mg, 1.758 mmol) and 4-dimethylaminopyridine (5.638 mmol, 0.044 mmol) were highly dried under diminished pressure for 20 min. Anhydrous pyridine (1 ml) was added at 0°C under anhydrous condition. The reaction mixture was stirred at 0°C for 12 hrs.

Then the reaction mixture was distilled *in vacuo* to remove pyridine. The residue was coevaporated with toluene (1 ml×3) in order to remove pyridine perfectly. The residue was purified with column chromatography (CHCl₃:CH₃OH=25:1, silica gel =10g, 400 mesh). The product was obtained as a white solid **7** (110 mg, 30%).

¹H-NMR (CDCl₃): 9.86 (s, 1H, H-3), 7.78-7.29 (m, 4H, aromatic), 7.48 (s, 1H, H-5), 5.94 (t, 1H, H-1'), 4.01-3.47 (m, 7H, H-2',3',4',5'), 2.41 (s, 3H, -CH₃), 1.31 (s, 6H, acetonide).

6-Aza-2'-Azido-2'-deoxy-3',5'-O-isopropylidene-2',3'-secouridine, 8

To the compound **7** (26 mg, 0.058 mmol) and sodium azide (7.82 mg, 0.12 mmol) was added DMF (1 ml) under anhydrous condition. The reaction mixture was stirred at 80°C for 4 hrs. Then the reaction mixture was distilled *in vacuo* to remove DMF. The residue was purified with column chromatography (CHCl₃:CH₃OH=15:1, silica gel=3g). The product was obtained as a transparent solid looked like an oil form **8** (17 mg, 67%).

¹H-NMR (CDCl₃): 9.65 (broad, s, 1H, H-3), 7.53 (s, 1H, H-5), 5.94 (t, 1H, H-1'), 4.01-3.46 (m, 7H, H-2',3',4',5'), 1.39 (s, 6H, acetonide).

IR (nujol): 2150 (N₃), 1680 (C=O).

6-Aza-2'-Azido-2'-deoxy-2',3'-secouridine, 9

To the compound **8** (60 mg, 0.199 mmol), 80 %-acetic acid (3 ml) and water (3 ml) were added. The reaction mixture was stirred at 80°C for 4 hrs. Then the reaction mixture was distilled *in vacuo* to remove water and acetic acid. The residue was coevaporated with toluene (1 ml×3) to remove acetic acid perfectly, and a vacuum pump was used for 1 hr. and then the residue was purified with column chromatography (CHCl₃:CH₃OH=10:1, silica gel=8g). The product was obtained as a transparent solid looked like an oil form **9** (54 mg, 90%).

¹H-NMR (DMSO-d₆): 9.85 (broad, s, 1H, H-3), 7.52 (s, 1H, H-5), 5.93 (t, 1H, H-1'), 4.46-4.21 (m, 2H, OH-3',5'), 3.89-3.56 (m, 7H, H-2',3',4',5').

IR (neat): 3385 (OH), 2111 (N₃), 1685 (C=O).

6-Aza-3',5'-p-nitrophenylphosphoryltri-oxy-2'-azido-2'-deoxy-2',3'-secouridine, 2

To a stirred solution of compound **9** (56.5 mg, 0.027 mmol) in dry acetonitrile (3 ml) was added pyridine (1 ml) and *p*-nitrophenyl phosphorodichloridate (80 mg, 0.311 mmol). This mixture was stirred at room temperature for 24 hrs. with the exclusion of moisture. The excess solvents were removed *in vacuo* and then the residue was purified with column chromatography (CHCl₃:CH₃OH=15:1). The product was obtained as a bright yellow solid **2** (32 mg, 34%).

¹H-NMR (DMSO-d₆): the more polar diastereomer: 11.35 (broad s, 1H, H-3), 8.26 (d, 2H, ortho position of *p*-nitrophenyl), 7.55-7.26 (m, 3H, meta position of *p*-nitrophenyl, H-5), 6.06 (t, 1H, H-1'), 5.00-3.55 (m, 7H, H-2',3',4',5'); the less polar diastereomer: 11.35 (broad s, 1H, H-3), 8.26 (d, 2H, ortho position of *p*-nitrophenyl), 7.45-7.26 (m, 3H, meta position of *p*-nitrophenyl, H-5), 6.03 (t, 1H, H-1'), 4.78-3.13 (m, 7H, H-2',3',4',5').

IR (KBr): 2111 (N₃), 1524, 1350 (NO₂).

6-Aza-3',5'-phosphoryltri-oxy-2'-azido-2'-deoxy-2',3'-secouridine ammonium salt, 1

Triester **2** (11 mg, 0.0242 mmol) was dissolved in 1,4-dioxane (1 ml) and to this solution was added

28%-NH₄OH (0.1 ml). After stirring overnight at room temperature, the excess solvents were removed *in vacuo* and the residue was purified with column chromatography (ethyl acetate:MeOH=7:3). The product was obtained as a white solid **1** (6 mg, 67%).

¹H-NMR (DMSO-d₆): 11.36 (broad, s, 1H, H-3), 8.26 (s, 1H, H-5), 5.84 (t, 1H, H-1'), 4.15-3.68 (t, 7H, H-2',3',4',5').

IR (nujol): 3300-3200 (NH₄⁺), 2111 (N₃).

Biological evaluation

Compound **1** and **2** were screened for activity against HSV-1, HSV-2, poliovirus and coxsackievirus by using Virus-induced CPE (cytopathic effect) inhibition assay method⁶.

The *in vitro* test results of these compounds showed no significant antiviral activity (EC₅₀>250 µg/ml), but no significant cytotoxic effect (CC₅₀>250 µg/ml) against Vero and Hela cell.

The nucleoside **9** is still undergoing evaluation.

ACKNOWLEDGEMENT

This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation 1990-1992 and RCNDD, Seoul National University.

LITERATURE CITED

1. Hong, K. A., Yang, J.-W., Chun, S. J., Ha, E.-Y., Kim, J. H., Chun, M. W. and Chung, W.-K.: A synthetic study on cyclic phosphate derivatives of seconucleosides as potential antiviral agents (1): Synthesis of 3',5'-cyclic phosphate of 2'-substituted secouridines and secoribavirins, *Arch. Pharm. Res.*, **14**, 30 (1991).
2. Kim, H. O., Chun, M. W. and Chng, W.-K.: Synthesis of acyclonucleoside (1), *Seoul Univ. J. Pharm. Sci.* **12**, 62 (1987).
3. Moon, H. R., Yang, J. W., Kim, M. H., Chun, M. W. and Chung, W.-K.: Synthesis of acyclonucleosides (3): Synthesis of ribavirin derivatives, *Yakhak Hoeji*, **33**, 361 (1989).
4. Lerner, L. M.: Preparation of trialcohols and some of their derivatives from nucleosides, *Carbohydr. Res.* **13**, 465 (1970).
5. Engels, J. and Schlareger, F. J.: Synthesis, structure, and reactivity of adenosine cyclic 3',5'-phosphate benzyl triesters, *J. Med. Chem.* **20**, 907 (1977).
6. Lee, C.-K., Rha, Z. S. and Kim, H. S.: *In vitro* evaluation of anti-Herpesviral activity of derivatives of nucleic acids and their precursors, *J. Kor. Soc. Vir.* **22**, 69 (1992).