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A Synthetic Study on Cyclic Phosphate Derivatives of Seconucleosides as Potential Antiviral Agents (I)
: Synthesis of 3′,5′-cyclic phosphates of 2′-substituted secouridines and secoribavirins

Kyong Aie Hong, Jae-Wook Yang, Soo Joon Chun, Eun-Young Ha, Joong Hyup Kim*, Moon Woo Chun and Won-Keun Chung
College of Pharmacy, Seoul National University, Seoul 151-742, Korea
**Korea Institute of Science and Technology, Seoul 131, Korea*
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Abstract □ The synthetic study of 3′,5-cyclic phosphates of 2′-substituted 2′,3′-secouridines and 2′,3′-secoribavirins toward development of new antiviral agents is described. These cyclic phosphates were synthesized from their respective 4-nitrophenyl 3′,5′-cyclic phosphate triesters. These triesters were prepared from the corresponding 2′-azido and 2′-bromo 2′,3′-seconucleosides.

Keywords □ Acyclonucleoside, cyclic phosphate of seconucleoside.

Acyclic nucleosides such as DHPG[1-3], acyclovir[4] are biologically active as antiviral agents (Fig. 1). The effectiveness of acyclic nucleosides as antiviral agents is highly dependent on whether they are substrates for phosphorylating enzymes. For example, DHPG is exceptionally potent against a broad range of herpes viruses[2]. In HSV-infected cells, DHPG is activated by phosphorylation by a virus-specified thymidine kinase to give DHPG monophosphate. The monophosphate is then converted by enzymes in the host cell to DHPG triphosphate, which, in turn, exerts the antiviral effect by inhibition of the viral DNA polymerase. The selectivity in activity is realized at two stages[3].

First, inasmuch as DHPG is not phosphorylated very effectively by host enzymes.

Secondly, host polymerases are less sensitive than the viral polymerase to the triphosphate.

But numerous thymidine kinase deficient strains of the viruses have been characterized and, in general, are resistant to the antiviral nucleosides like DHPG.

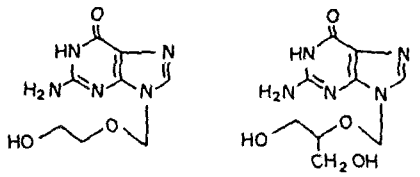
The low activity of the nucleosides against these viruses appears to be due to the low level of phosphorylation of the nucleosides by host enzymes.

Therefore, a number of phosphate esters of the nucleosides have been synthesized with the hope of bypassing the phosphorylation step[3,5-7].

The present work describes the chemical synthesis of 3′,5′-cyclic monophosphates of 2′-azido-2′-deoxy-2′,3′-secouridine(**1**) and 2′-azido-2′-deoxy-2′,3′-secoribavirin (**5**) aming improved activity as efficient antiviral agents.

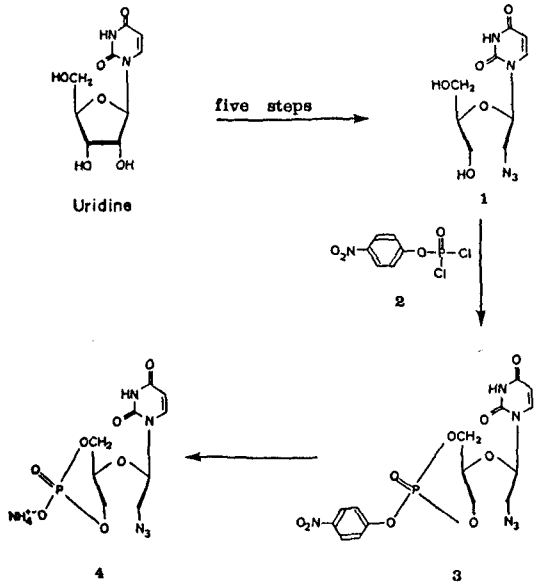
The chemical synthesis of 2′-azido-2′-deoxy-2′,3′-secouridine (**1**) and 2′-azido-2′-deoxy-2′,3′-secoribavirin(**5**) has previously been reported[8,9].

In the present study, **1** and **5** were treated with 4-nitrophenyl phosphorodichloridate[7,10] (**2**) in dry acetonitrile in the presence of pyridine at room temperature to yield 3′,5′-cyclic p-nitrophenyl phosphate (**3**, **6**) of **1** and **5** as diastereomeric mixtures[11]

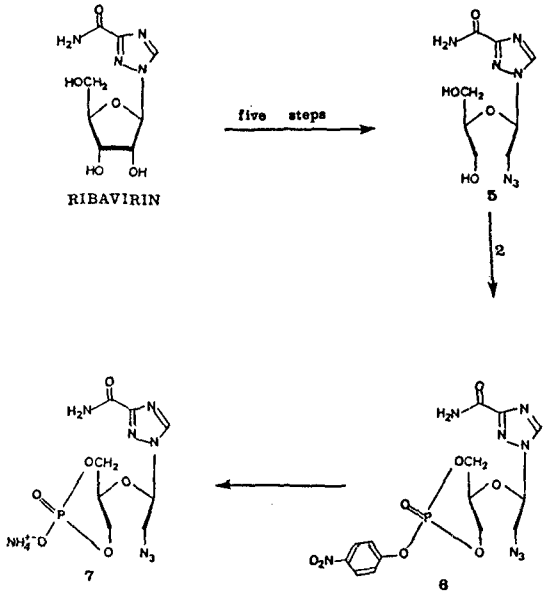


Acyclovir DHPG

Fig. 1



Scheme 1



Scheme 2

in excellent yields (Scheme 1, 2). When we treated each diastereomer with ammonia after separation, identical products were obtained from each diastereomer. (However, we couldn't separate each diastereomer because it was too difficult to separate in the case of ribavirin derivatives.) But there was difference in the rate of ammonolysis of two diastereomers. 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.

We also tried cyclic phosphorylation of 2'-bromo analogues (**8, 11**) by same method (Scheme 3). But the result was not satisfactory. 3',5'-Cyclic p-nitrophenyl monophosphate of 2'-bromo analogues decomposed to undesired byproducts. Therefore we obtained them in poor yield in comparison with the case of 2'-azido analogues.

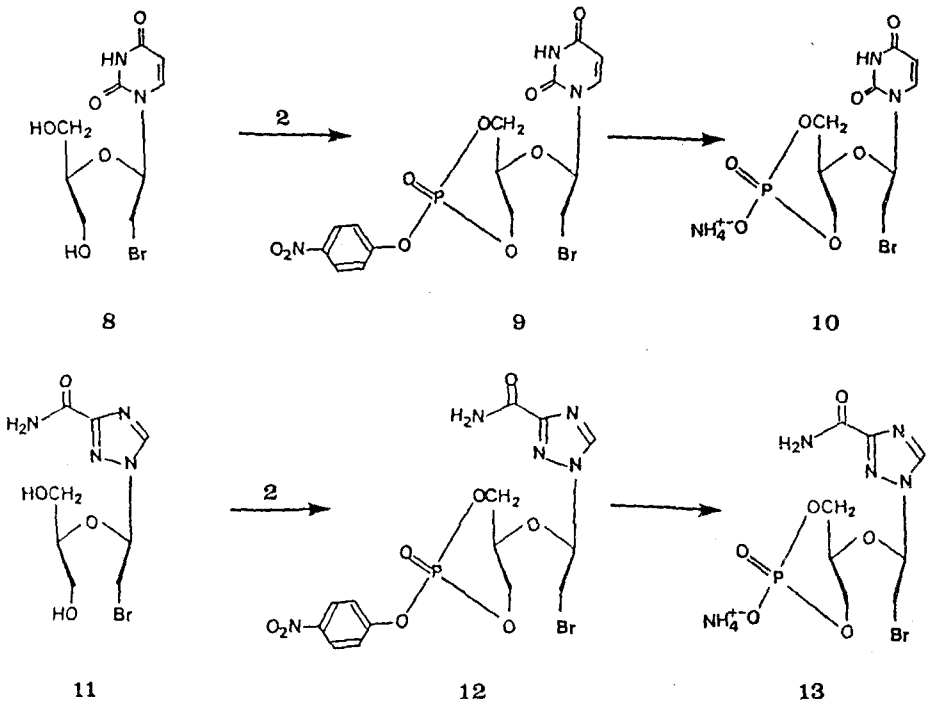
EXPERIMENTAL

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 frequences are given in reciprocal centimeters.

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.

3',5'-p-nitrophenylphosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secouridine (3)

To a stirred solution of 2'-azido-2'-deoxy-2',3'-secouridine (**1**, 311 mg, 1.15 mmol) in dry acetonitrile (5 m*l*) was added pyridine (1 m*l*) and p-nitrophenyl phosphorodichloridate (**2**, 440.8 mg, 1.72 mmol). This mixture was stirred at room temperature for 24 hours with the exclusion of moisture. The excess solvents were removed *in vacuo* and then, the residue was purified with column chromatography ($CHCl_3$/MeOH=15/1). The product was obtained as a bright yellow solid. Yield: 430 mg (83%). IR (KBr): 2111 (N_3), 1693 (pyrimidinone), 1524, 1350 (NO_2). ^{1}H-NMR (DMSO-d_6): more polar diastereomer 11.35 (brs. 1H, −NH−), 8.26 (d, 2H, J=8.8, ortho position of p-nitrophenyl), 7.55-7.26 (m, 3H, meta position of p-nitrophenyl and H-6), 6.06 (t, 1H, J=5.2, H-1'), 5.88 (d, 1H, J=8, H-5), 5.00-3.55 (m, 7H, H-2',3',4',5'); less polar diastereomer; 11.35 (brs, 1H, −NH−), 8.26 (d, 2H, J=8.8, ortho position of p-nitrophenyl), 7.45-7.26 (m, 3H, meta posi-



Scheme 3

tion of p-nitrophenyl and H-6), 6.03 (t, 1H, J=5.2, H-1'), 5.82 (d, 1H, J=8, H-5), 4.78-4.15 (m, 5H, H-3',4',5'), 3.15 (d, 2H, J=5.2, H-2'). Anal. Calcd. for $C_{15}H_{15}N_6O_9P$: C 39.65, H 3.33, N 18.50; Found: C 39.39, H 3.33, N 18.19.

3',5'-phosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secouridine ammonium salt (4)

Triester (**3**, 192 mg, 0.43 mmol) was dissolved in p-dioxane (5 m*l*) and to this solution was added 28% NH_4OH (0.5 m*l*). After stirring overnight at room temperature. The excess solvents were removed *in vacuo*. and the residue was purified with column chromatography (EtOAc/MeOH=7/4). The product **4** was obtained as a white solid. Yield: 109 mg (73%). IR (nujol): 3300-3200 (NH_4^+ st br), 2106 (N_3), 1685 (pyrimidinone). ^{1}H-NMR (DMSO-d_6): 11.36 (brs, 1H, −NH−), 7.66 (d, 1H, J=8, H-6), 5.84 (t, 1H, J=5.8, H-1'), 5.66 (d, 1H, J=8, H-5), 4.10-3.57 (m, 7H, H-2',3',4',5'). Anal. Calcd. for $C_9H_{15}N_6O_7P$: C, 30.86, H 4.32, N 24.00; Found: C 30.22, H 4.32 N 23.98.

3',5'-p-nitrophenylphosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secoribavirin (6)

To a stirred solution of diol (**5**, 180 mg, 0.63 mmol) in dry acetonitrile (5 m*l*) were added dry pyridine (0.66 m*l*) and p-nitrophenylphosphorodichloridate (**2**, 240.1 mg, 0.95 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 ($CHCl_3$/MeOH=20/1). The product **6** was obtained as a yellow solid. Yield: 235 mg (80%). IR (nujol): 2110(N_3), 1520, 1350 (NO_2), 1690 (C=O). ^{1}H-NMR ($CDCl_3$+DMSO-d_6): 8.76 (s, 1H, H-5), 8.27 (d, J=8.8, 2H, ortho position of p-nitrophenyl), 7.50-7.00 (m, 4H, meta position of p-nitrophenyl and amide-H) 6.08 (t, J=4.8, 1H, H-1'), 4.90-3.78 (m, 7H, H-2',3',4',5'). Anal. Calcd. for $C_{14}H_{15}N_8O_8P$: C 37.00, H 3.30, N 24.67; Found: C 37.12, H 3.43, N 24.59.

3',5'-phosphoryltrioxy-2'-azido-2'-deoxy-2',3'-secoribavirin ammonium salt (7)

Triester (**6**, 60 mg, 0.128 mmol) was dissolved in p-dioxane (4 m*l*) and to this solution was added 28% NH_4OH (0.5 m*l*). After stirring overnight at

room temperature, the excess solvents were removed *in vacuo*, and the residue was purified with column chromatography (EtOAc/MeOH=5/3). The product **7** was obtained as a white solid. Yield: 28 mg (60%). IR (nujol): 3300-3200 (NH_4^+), 2110 (N_3), 1685 (C=O). ^{1}H-NMR (DMSO-d_6): 8.89 (s, 1H, H-5), 7.73 (d, 2H, amide-H), 6.01 (t, 1H, H-1'), 4.22-3.18 (m, 7H, H-2',3',4',5'). Anal. Calcd. for $C_8H_{15}N_8O_6P$: C 30.86, H 4.29, N 32.00; Found: C 30.82, H 4.31, N 31.96.

3',5'-p-nitrophenylphosphoryltrioxy-2'-bromo-2'-deoxy-2',3'-secouridine (9)

To a stirred solution of diol **8** (97 mg, 0.31 mmol) in dry acetonitrile (3 m*l*) were added dry pyridine (0.33 m*l*) and p-nitrophenylphosphorodichloridate (**2**, 120.6 mg, 0.47 mmol). This mixture was stirred at room temperature for 24 hours with the exclusion of moisture. The excess solvents were removed *in vacuo* and the residue was purified with column chromatography ($CHCl_3$/MeOH=15/1). The product **9** was obtained as a white solid. Yield: 89 mg (59%). IR (nujol): 1688 (C=O), 1521, 1360 (NO_2). ^{1}H-NMR (DMSO-d_6): the more polar diastereomer, 11.40 (brs, 1H, −NH−), 8.32 (d, J=8.8, 2H, ortho position of p-nitrophenyl), 7.80 (d, J=8, 1H, H-6), 7.53 (d, J=8.8, 2H, meta position of p-nitrophenyl), 6.10 (t, J 6.3, 1H, H-1'), 5.76 (d, J=8, 1H, H-5), 3.96-5.44 (m, 7H, H-2',3',4',5'); the less polar diastereomer, 11.41 (brs, 1H, -NH-), 8.32 (d, J=8.8, 2H, ortho positoin of p-nitrophenyl), 7.78 (d, J=8, 1H, H-6), 7.52 (d, J=8.8, 2H, meta position of p-nitrophenyl), 6.05 (t, J=6.4, 1H, H-1'), 5.70 (d, J=8, H-5), 5.04-4.0 (m, 5H, H-3',4',5'), 3.88 (d, J=6.4, $-CH_2Br$). Anal. Calcd. for $C_{15}H_{15}N_3O_9BrP$: C 36.60, H 3.07, N 8.54; Found: C 35.89, H 3.19, N 8.20.

3',5'-phosphoryltrioxy-2'-bromo-2'-deoxy-2',3'-secouridine ammonium salt (10)

Triester **9** (95.2 mg, 0.192 mmol) was dissolved in p-dioxane (4 m*l*) and to this solution was added 28% -NH_4OH (0.3 m*l*). After stirring overnight at room temperature, the excess solvents were removed *in vacuo*, and the residue was purified with column chromagraphy (EtOAc/MeOH=7/4). The product **10** was obtained as a white solid. Yield: 14.8 mg (20%). IR (nujol): 3300-3200 (NH_4^+), 1685 (C=O). ^{1}H-NMR(DMSO-d_6): 11.30 (brs, 1H,−NH−), 7.59 (d, 1H, J=8, H-6), 5.89 (t, 1H, J=5.8, H-1'), 5.69 (d, 1H, J=8, H-5), 4.25-3.27 (m, 7H, H-2',3',4',5'). Anal. Calcd. for $C_9H_{15}N_3O_7BrP$: C 27.84, H 3.87, N 10.82; Found: C 27.65, H 3.92, N 10.72.

3',5'-p-nitrophenylphosphoryltrioxy-2'-bromo-2'-deoxy-2',3'-secoribavirin (12)

To a stirred solution of diol **11** (200 mg, 0.62 mmol) in dry acetonitrile (5 m*l*) were added dry pyridine (1 m*l*) and p-nitrophenylphosphorodichloridate (**2**, 236.3 mg, 0.92 mmol). This mixture was stirred at room temperature for 24 hours with the exclusion of moisture. The excess solvents were removed *in vacuo* and then, the residue was purified with column chromatography($CHCl_3$/MeOH=10/1). The product **12** was obtained as a white solid. Yield: 149 mg (48%). IR (nujol): 1690 (C=O), 1520, 1350 (NO_2). ^{1}H-NMR (DMSO-d_6): 8.93 (s, 1H, H-5), 8.32 (d, J=8.8, 2H, ortho position of p-nitrophenyl), 7.73 (d, 2H, amide-H), 7.52 (d, J=8.8, 2H, meta position of p-nitrophenyl), 6.25 (t, J=6.4, 1H, H-1'), 5.00-4.21 (m, 5H, H-3',4',5'), 4.04 (d, J=6.4, 2H, H-2'). Anal. Calcd. for $C_{14}H_{15}N_5O_8BrP$: C 34.15, H 3.05, N 14.23; Found: C 33.99, H 3.09, N 14.11.

3',5'-phosphoryltrioxy-2'-bromo-2'-deoxy-2',3'-secoribavirin ammonium salt (13)

Triester **12** (120 mg, 0.24 mmol) was dissolved in p-dioxane (5 m*l*) and to this solution was added 28% -NH_4OH(1 m*l*). After stirring overnight at room temperature, the excess solvents were removed *in vacuo*, and the residue was purified with column chromatography (EtOAc/MeOH=5/3). The product **13** was obtained as a white solid. Yield: 39 mg (40 %). IR (nujol): 1690 (C=O), 3300-3200 (NH_4^+). ^{1}H-NMR (DMSO-d_6): 8.91 (s, 1H, H-5), 7.75 (d, 2H, amide-H), 6.05 (t, J=5.5, 1H, H-1'), 4.25-3.13 (m, 7H, H-2',3',4',5'). Anal. Calcd. for $C_8H_{15}N_5O_6BrP$: C 27.47, H 3.87, N 18.04; Found: C 27.40, H 3.95, N 18.01.

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