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Synthetic Nucleosides and Nucleotides. XIX.[1] Synthesis of 3′-Deoxycytidine 5′-Triphosphate and Related 3′-Deoxyribonucleotides from Cordycepin

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The pyrimidine 3′-deoxyriboside 5′-triphosphates, pyrimidine counterparts of cordycepin 5′-triphosphate, (1) were conveniently synthesized from cordycepin as potential deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase inhibitors. For the synthesis of 3′-deoxycytidine, *p*-chlororobenzoylation of 1 followed by acetolysis afforded an anomeric mixture of 1-*O*-acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (3) in good yield. Condensation of 2,4-bis-trimethylsilyl-N_4-acylcytosine (**4a**, **b**) with **3** in the presence of stannic chloride gave fully acylated 3′-deoxycytidine (**5**) in excellent yield. The protecting group was removed to give 3′-deoxycytidine (**6**), which on phosphorylation gave 3′-deoxycytidine 5′-monophosphate (**7**), a key intermediate in the present study. Deamination of **7** with nitrous acid afforded 3′-deoxyuridine-5′-monophosphate (**8**). Additionally, 2,4-bis-trimethylsilyloxy-5-methyl- or ethylpyrimidine (**4c, d**) was coupled with **3** in a similar manner to give fully acylated 5-methyl- (**5c**) or 5-ethyl-3′-deoxyuridine (**5d**). The 5′-monophosphates were converted to the corresponding 5′-triphosphates by the phosphoroimidazolidate method.

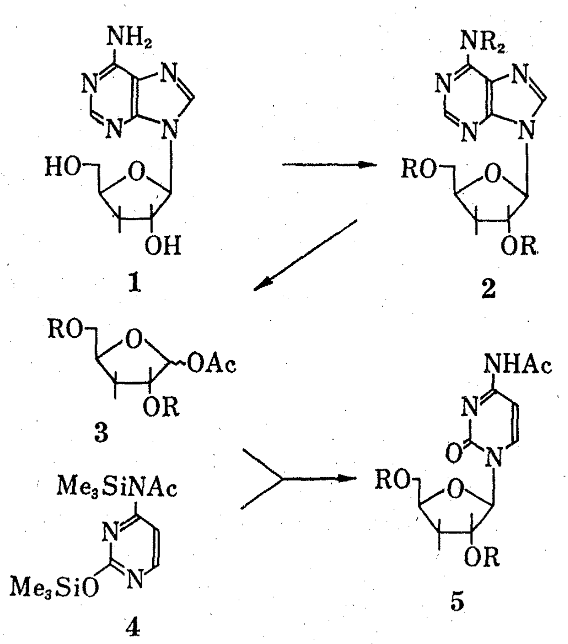
Keywords——cordycepin; 3′-deoxycytidine; 3′-dCTP; 3′-dUTP; 3′-dTTP; RNA polymerase inhibitor

Cordycepin (3′-deoxyadenosine) (**1**) is a nucleoside antibiotic isolated from culture filtrates of *Cordyceps militaris* and *Aspergillus nidulans*.[2] This antibiotic is cytotoxic to both prokaryotes and eukaryotic cells. Cordycepin has been shown in both bacterial and mammalian cells to inhibit the activities of several enzymes in the purine biosynthetic pathway,[3] deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) synthesis,[4] and polyadenylate synthesis.[5] *In vitro*s tudies have shown that cordycepin 5′-triphosphate (3′-dATP) is the active form of the antibiotic cordycepin *in vivo* and that it inhibits DNA-dependent RNA polymerase from *K. lysodeikticus*[6] and yeast.[7]

We previously showed that 1-*β*-D-arabinofuranosylthymine 5′-triphosphate (Ara TTP),[8—9] and 9-*β*-D-arabinofuranosylguanine 5′-triphosphate (Ara GTP)[10] have strong inhibitory effects on DNA-dependent DNA polymerase from murine tumor cells and RNA-dependent DNA polymerase from oncorna virus, as do AraCTP and AraATP. These results prompted us to synthesize 3′-deoxycytidine 5′-triphosphate (3′-dCTP) and related pyrimidine 3′-deoxyribonucleoside 5′-triphosphates (3′-dNTP) as pyrimidine counterparts of cordycepin 5′-triphosphate, and to study the influence of these analogues on RNA synthesis *in vitro*.

This paper reports the chemical synthesis of 3′-dCTP and related nucleotides starting from the antibiotic cordycepin. The key nucleoside 3′-deoxycytidine (**6**) was first synthesized from D-xylose[11] more than 10 steps, and an alternative method[12] was also very long. Our present method is especially useful, as cordycepin (**1**) is readily available and can serve as a convenient starting compound for the preparation of 3′-deoxycytidine (**6**). *p*-Chlorobenzoylation of **1** followed by acetolysis of tetrabenzoylcordycepin (**2**) afforded an anomeric mixture of 1-*O*-acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (**3**). Condensation of **3** with the trimethylsilylated derivative of N_4-acetylcytosine (**4a**) or N_4-*n*-butyrylcytosine (**4b**) in acetonitrile in the presence of stannic chloride gave the fully acylated nucleoside, N_4-acetyl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (**5a**), mp 203—204°C (quantitative) or N_4-*n*-

butyryl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (**5b**), mp 203—205°C (91%). Saponification of these compounds afforded known 3′-deoxycytidine[11,12] (**6**). β-Configuration of **6** was confirmed by comparison of its circular dichroism (CD) spectrum with that of 2′-deoxycytidine. 2,4-Bis-trimethylsilyloxy-5-methyl- and 5-ethylpyrimidine (**4c**, **d**) were also coupled with **3** to give 2′,5′-di-*O*-*p*-chlorobenzoyl-5-methyl-3-deoxyuridine (**5c**), mp 143—144.5°C (53%) and 2′,5′-di-*O*-*p*-chloro-benzoyl-5-ethyl-3′-deoxyuridine (**5d**), mp 147—149°C (70%), respectively. Compound **6** was phosphorylated to 3′-deoxycytidine 5′-monophosphate (3′-dCMP) (**7**) using tetrachloropyrophosphate in acetonitrile[13] or phosphorus oxychloride in triethyl phosphate.[14] Compound **7** was deaminated with sodium nitrite in acetic acid followed by column chromatography on diethylaminoethyl (DEAE)-cellulose to afford 3′-deoxyuridine 5′-monophosphate (3′-dUMP) (**8**). Compound **5c** was saponified to the free nucleoside, 3′-deoxythymidine, which was then phosphorylated to the 5′-monophosphate. These monophosphates were converted to their 5′-triphosphates by using the phosphoromorpholidate method[15] or the phosphoroimidazolidate method.[16] These 3′-deoxyribonucleotide analogs showed strong inhibitory effects on DNA-dependent RNA polymerases I and II from *D. discoideum* cells[17] and from cherry salmon (*Oncorhynchus masou*) liver.[18]



R=*p*-chlorobenzoyl
Ac=acetyl

Chart 1

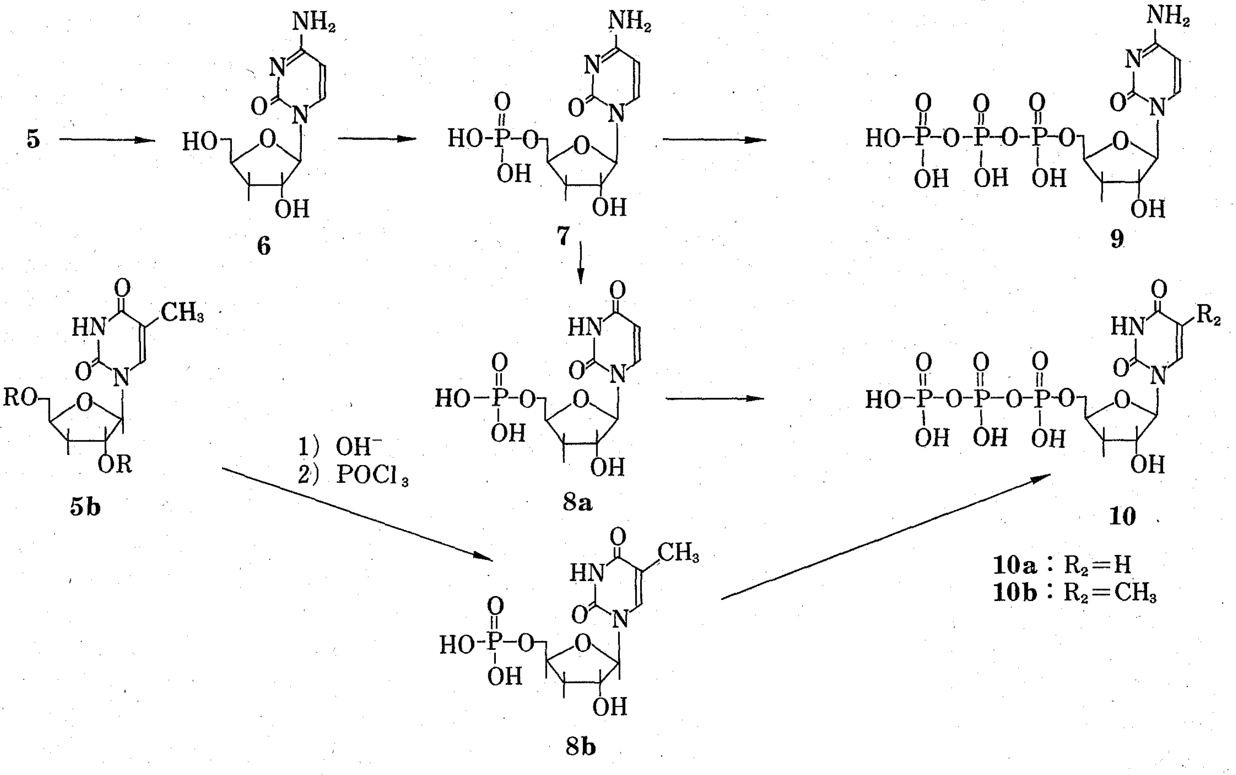


Chart 2

Experimental

N_6,N_6,2′,5′-Tetra-*p*-chlorobenzoylcordycepin (2)——*p*-Chlorobenzoyl chloride (20.9 g, 119.4 mmol) was added dropwise to a suspension of cordycepin (1) (5 g, 19.9 mmol) in 200 ml of anhydrous pyridine under cooling in an ice-bath. The mixture was stirred at room temperature for 2 h and kept at 37°C overnight. The clear solution was concentrated to a small volume (*ca.* 40 ml) and the residue was mixed with water (500 ml) to give a colorless crystalline mass. The suspension was filtered and the solid was washed well with 5% aqueous sodium bicarbonate and water and air-dried to give 16.4 g of product. This crude material was crystallized from ethanol–chloroform to give fine needles. mp 220—224°C. *Anal.* Calcd for $C_{38}H_{25}Cl_4N_5O_7$: C, 56.67; H, 3.13; Cl, 17.61; N, 8.69. Found: C, 56.61; H, 3.20; Cl, 17.78; N, 8.79.

1-*O*-Acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (3)——Ten grams of compound **2** was suspended in a mixture of acetic acid (80 ml) and acetic anhydride (20 ml). To this suspension, concentrated sulfuric acid (10 ml) was added dropwise below 10°C under cooling in an ice-bath. A clear solution was obtained after stirring for 2 h at room temperature and the *p*-chlorobenzoyladenine began to separate from the reaction mixture as a colorless solid after 24 h. The precipitates were removed by suction filtration and washed with acetic acid (10 ml). The filtrate and washings were combined, neutralized with anhydrous sodium acetate (56 g, equivalent to sulfuric acid used), and concentrated to dryness. The residue was treated with water (200 ml) and extracted with chloroform (200 ml × 3). The chloroform extracts were combined, washed successively with aqueous saturated sodium bicarbonate (100 ml × 3) and water (100 ml × 3), dried over anhydrous magnesium sulfate, and concentrated to give a crude anomeric mixture of 1-*O*-acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (**3**) (4.0 g) as a colorless gum. This material contained a small amount of acyclic sugar derivative as a by-product. The crude material was dissolved in dry benzene and applied to a column of silica gel (75 g), and the column was eluted with benzene–ethyl acetate (19: 1). The acyclic sugar derivative was eluted first and **3** was eluted as the main peak. The fractions containing the desired **3** were combined and evaporated to dryness to afford a colorless solid. This compound was used directly in the next step.

N_4-Acetyl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (5a) and N_4-*n*-butyryl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (5b)——Trimethylsilylated N_4-acetylcytosine (**4a**) derived from 1.5 g of N_4-acetylcytosine was mixed with the 1-*O*-acetyl sugar (**3**) (4.2 g) in dry acetonitrile (50 ml). Stannic chloride (1.3 eq) was added to the solution and the mixture was stirred at room temperature overnight. Then, the mixture was evaporated under reduced pressure, the residue was mixed with saturated aqueous sodium bicarbonate (50 ml), and the mixture was evaporated to dryness. The protected nucleoside was extracted from the residue with boiling acetone (50 ml × 3). The solvent was removed from the extracts under reduced pressure and the residual gum was crystallized from 2-propanol to give N_4-acetyl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (**5a**) as colorless needles, mp 202—203°C. Quantitative yield. UV λ_{max}^{EtOH} (nm): 245 and 297. *Anal.* Calcd for $C_{25}H_{21}Cl_2N_3O_7$: C, 54.95; H, 3.88; Cl, 12.98; N, 7.96. Found: C, 54.76; H, 3.85; Cl, 12.90; N, 7.65. A similar experiment using N_4-*n*-butyrylcytosine (**4b**) gave N_4-*n*-butyryl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (**5b**) in 91% yield. mp 203—205°C. UV λ_{max}^{EtOH} (nm): 245 and 298. *Anal.* Calcd for $C_{27}H_{25}Cl_2N_3O_7$: C, 55.78; H, 4.40; Cl, 12.34; N, 7.32. Found: C, 56.08; H, 4.56; Cl, 12.34; N, 7.10.

3′-Deoxycytidine 5′-Monophosphate (7)——N_4-Acetyl-2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (**5a**) (3.5 g) was treated with 0.05 M methanolic sodium methoxide (100 ml) and the mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in water (100 ml). The aqueous solution was extracted with chloroform (50 ml × 4) to remove methyl *p*-chlorobenzoate. Sodium ion was removed by treatment of the aqueous solution with Dowex 50 (H^+-form) and the resin was filtered off. The filtrate was evaporated and the residue was dissolved in a small amount of water, applied to a column of Dowex 1 (OH^--form), and eluted with water. The eluate was evaporated and the residue was dried and crystallized from a minimum amount of ethanol to give 3′-deoxycytidine (**6**)[11,12] (980 mg). mp 213—215°C. UV $\lambda_{max}^{H_2O}$ (nm): 271 ($\varepsilon=9100$); $\lambda_{max}^{0.01N\ HCl}$ (nm): 278 ($\varepsilon=12800$). CD in H_2O (nm): $[\theta]_{271}=+18700$.

3′-Deoxycytidine (**6**) (100 mg) was suspended in dry acetonitrile (3 ml) and phosphorylated with tetrachloropyrophosphate (7 μl) by stirring the mixture overnight at room temperature. The phosphorylated product was purified by ion-exchange column chromatography on Dowex 1 (formate form) (1.2 × 30 cm). Elution was performed stepwise with 0.01 M formic acid (70 ml) and 0.05 M formic acid (130 ml). The desired 3′-deoxycytidine 5′-monophosphate (**7**) was eluted with 0.05 M formic acid. Yield, 1020 A_{280} units in 0.01 N HCl. (74%). UV $\lambda_{max}^{H_2O}$ (nm): 271 ($\varepsilon=9100$); $\lambda_{max}^{0.01N\ HCl}$ (nm): 278 ($\varepsilon=12800$). Phosphate analysis: $\varepsilon(P)=13300$. Paper chromatography, $Rf=0.67$ (Solvent A). $Rf=0.34$ (Solvent B). Solvent A, isobutyric acid–0.5 M NH_4OH (5: 3, v/v). Solvent B, *n*-propanol–conc. NH_4OH–H_2O (7: 2: 1, v/v/v). Paper electrophoretic mobilities in 0.05M triethylammonium bicarbonate, pH 8.5 (System A), 600 V for 40 min. $R_{2'\text{-dCMP}}=1.03$; in 30 mM potassium citrate, pH 4.1 (System B), $R_{2'\text{-dCMP}}=0.98$.

3′-Deoxyuridine 5′-Monophosphate (8)——Sodium nitrite (138 mg) was added to a solution of **7** (21 mg, as free acid) in 50% acetic acid (2 ml) and the mixture was stirred for 4 h at room temperature. The UV absorption maximum of the mixture changed from 280 nm to 260 nm during the reaction, showing the conversion of the 3′-deoxycytidine residue to a 3′-deoxyuridine residue. The solvent was removed and the residue was dissolved in 100 ml of water. The solution was applied to a column of DEAE-cellulose (bicarbonate form) (1.2×40 cm). Elution was performed with a linear gradient of 0 to 0.2 M triethylammonium bicarbonate (pH 8.0, 500 ml each). The fractions containing the nucleotide were combined and the solvent was evaporated off. The residue was further purified by preparative paper chromatography using 40 cm×40 cm Toyo No. 51A paper (Solvent B). The band corresponding to 3′-deoxyuridine 5′-monophosphate (**8**) was cut off and eluted with water and the eluate was concentrated. Yield 67% based on UV absorbance at 260 nm. UV $\lambda_{max}^{H_2O}$ (nm): 260. Phosphate analysis using ε in the literature.[12] Calculated ε(P)=10600; Found, ε(P)=10200. Paper chromatography, *Rf*=0.75 (Solvent A), 0.34 (Solvent B). Paper electrophoretic mobilities: R_{dTMP}=1.05 (System A) R_{dTMP}=0.99 (System B).

2′,5′-Di-*O*-*p*-chlorobenzoyl-5-methyl-3′-deoxyuridine (5b) and 2′,5′-di-*O*-*p*-chlorobenzoyl-5-ethyl-3′-deoxyuridine (5c)——Stannic chloride (1.5 eq to 3) in 10 ml of acetonitrile was added to a solution of 3 (1.34 g 2.9 mmol) and 2,4-bis-trimethylsilyloxy-5-methylpyrimidine [derived from 360 mg (2.86 mmol) of thymine] in 30 ml of anhydrous acetonitrile under stirring. The clear solution was stirred at room temperature overnight. The mixture was treated with sodium bicarbonate (2 g) and water (10 ml). The neutralized solution was filtered through Celite and the filter cake was washed with 60 ml of hot chloroform. The organic layer of the filtrate was separated, concentrated to a small volume and applied to a column of silica gel (20 g). Elution was performed with chloroform–methanol (9: 1, v/v). The main peak was collected and the solvent was evaporated off. The residue was crystallized from methanol–isopropyl ether to give 0.78 g (53%) of **5b**, mp 143—144.5°C. *Anal.* Calcd for $C_{24}H_{20}Cl_2N_2O_7$: C, 55.50; H, 3.89; N, 5.40; Cl, 13.65. Found: C, 55.30; H, 3.87; N, 5.13; Cl, 13.79. A similar experiment using 2,4-bis-trimethylsilyloxy-5-ethylpyrimidine afforded crystalline **5c** in 70% yield, mp 147—149°C. *Anal.* Calcd for $C_{25}H_{22}Cl_2N_2O_7$: C, 56.29; H, 4.17; N, 5.25; Cl, 13.29. Found: C, 56.46; H, 4.13; N, 5.37; Cl, 13.41.

5-Methyl-3′-deoxyuridine 5′-Monophosphate (8b) (3′-dTMP)——Compound **5b** (548 mg) was treated with 170 mg of sodium methoxide in 50 ml of anhydrous methanol at room temperature for 3 h. After removal of the solvent by evaporation, the residue was dissolved in water (50 ml) and neutralized with Dowex 50 (H^+-form). The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel (10 g) column. Elution was performed with chloroform–methanol (4: 1, v/v). A UV absorbing peak was collected and concentrated to give free nucleoside. This compound was phosphorylated with phosphorus oxychloride in triethyl phosphate followed by purification using DEAE-cellulose column chromatography. Yield 67% based on UV absorbance at 268 nm.

General Procedure for the Synthesis of 3′-Deoxyribonucleoside 5′-Triphosphates——3′-Deoxyribonucleoside 5′-monophosphate (10 mg, as free acid) was dissolved in water (2 ml) containing 3 equivalent of freshly distilled tri-*n*-butylamine. The resulting solution was evaporated to dryness. Repeated evaporation with anhydrous dimethylformamide (2 ml×3) was carried out to remove traces of water. The residue was dissolved in dry dimethylformamide (1 ml) /and mixed with *N*,*N*′-carbonyldiimidazole (25 mg, 5 eq). The mixture was stirred for 2 h, then excess reagent was quenched with 3 μl of methanol, and tri-*n*-butylammonium pyrophosphate in dimethylformamide (0.25 ml, 0.5 mmol/ml) was added to the reaction mixture. The solution was stirred at room temperature for 2 h. The solvent was diluted with 40 ml of water and applied to a column of DEAE-cellulose (bicarbonate form, 0.9×30 cm). Elution was performed with a linear gradient from water (250 ml) to 0.4 M triethylammonium bicarbonate, pH 8.5 (250 ml). The fractions containing the triphosphate were combined and concentrated to give the 5′-triphosphate. Yield are summarized in Table I. The sodium salt was obtained by treatment of this material with sodium iodide in methanol and precipitation by addition of 2.5 volumes of acetone. The properties of the resulting 5′-triphosphates are also summarized in Table I.

TABLE I. Properties of 3′-Deoxyribonucleoside 5′-Triphosphates

Compound	UV, λ max	(nm) ε	ε (P)[a]	*Rf* values (PPC)			Paper electrophoresis (System B)[e]	Yield (%)
				A[b]	B[c]	D[d]		
3′-dCTP (**9**)	$\lambda_{max}^{H_2O}$ 270	(13000)	4600	0.47	0.53	0.77	$R_{2'-dCTP}$=0.98	78
	$\lambda_{max}^{0.01\,N\,HCl}$ 281							
3′-dUTP (**10a**)	$\lambda_{max}^{H_2O}$ 263	(10000)	3300	0.18	0.21	0.37	R_{UTP}=0.97	77
3′-dTTP (**10b**)[f]	$\lambda_{max}^{H_2O}$ 268	(9600)	3200	0.20	0.24	0.41	$R_{2'-dTTP}$=1.08	75

a) Phosphate analysis, Found.
b) Isobutyric acid–0.5 M NH_4OH (5: 3, v/v).
c) *n*-Propanol– c.NH_4OH– H_2O (7: 2: 1, v/v).
d) Ethanol– 1 M ammonium acetate (pH7.5) (1: 1, v/v).
e) 30 mM potassium citrate, pH 4.1, 600 V for 40 min.
f) 5-Methyl-3′-deoxyuridine 5′-triphosphate.

References and Notes

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