Notes

Chem. Pharm. Bull. 23(5)1146—1151(1975)

UDC 547.963.32.057

Synthetic Nucleosides and Nucleotides. IX.[1] Synthesis of 2′,5′- and 3′,5′-Di-O-tritylcytidine and the Related Compounds *via* Thiation

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(Received June 5, 1974)

Thiation of the 2′,5′-di-O-trityl-3′-O-acetyluridine or 3′,5′-di-O-trityl-2′-O-acetyluridine with phosphorus pentasulfide in refluxing toluene containing 20—30% pyridine afforded corresponding "4-thio" derivative in good yield. These "4-thio" derivatives were converted to the cytidine counterparts by the reaction with methanolic ammonia. 2′,5′-Di-O-trityl-3′-O-methanesulfonyluridine was also converted to cytidine derivative by similar manner. In addition, phosphorylation and dinucleoside monophosphate synthesis using titled compounds were described.

The usefulness of 2′,5′- or 3′,5′-di-O-tritylated ribonucleoside has led to synthesis of number of sugar modified derivatives including nucleotides.[3]

In an earlier paper,[4] we have described the thiation of 5′-O-trityl-2′,3′-di-O-acetyluridine in good yield of the corresponding 4-thio derivative.

In this paper we describe an extension of this work to di-tritylated uridine derivatives and the use of these thiated compounds as intermediates in facile synthesis of 2′,5′-di-O-tritylcytidine[5–7] 3′,5′-di-O-tritylcytidine[6–7] and related compounds.

We use 2′,5′-di-O-trityluridine[8] (II) and 3′,5′-di-O-trityluridine[9] as starting materials.

Treatment of II with acetic anhydride in anhydrous pyridine gave the 3′-O-acetylated derivative IV in almost quantitative yield (Chart 1), which was then refluxed with phosphorus pentasulfide (P_2S_5) in dry toluene containing 20—30% pyridine to give the corresponding 4-thio derivative (V) in 77—83% yield. The yield of V depend on the ratio of toluene to pyridine, as can be seen from Table I. Compound V was deacetylated with 0.5N methanolic sodium methoxide to afford 2′,5′-di-O-trityl-4-thiouridine (VI) in 85% yield. Compound IV and resulting compound V were easily converted to 2′,5′-di-O-tritylcytidine (VII) by treatment with methanolic ammonia at 95—100° for 10 hr. Detritylation of VII with 98% formic acid at room temperature gave cytidine. The structure of the product VII is supported by the fact that by converting the treatment of VII with *n*-amyl nitrite in dimethyl sulfoxide in the presence of acetic acid gave II. The total yield of VII from the starting material II was around 60—65%.

1) Part VIII of this series: M. Saneyoshi, *Chem. Pharm. Bull.* (Tokyo), **19**, 493 (1971). Part of this work was presented at the Annual Meeting of the Pharmaceutical Society of Japan at Nagoya, April 1969.
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3) *a*) R.H. Hall and R. Thedford, *J. Org. Chem.*, **28**, 1506 (1963); *b*) T. Ukita, Y. Takeda, and H. Hayatsu, *Chem. Pharm. Bull.* (Tokyo), **12**, 1503 (1964); *c*) P.R. Taylor and R.H. Hall, *J. Org. Chem.*, **29**, 1078 (1964); *d*) Y. Furukawa, K. Kobayashi, Y. Kanai, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **13**, 1273 (1965); *e*) Y. Mizuno, T. Ito, and H. Tagawa, *Chem. & Ind.*, **1965**, 1498.
4) M. Saneyoshi and F. Sawada, *Chem. Pharm. Bull.* (Tokyo), **17**, 181 (1969).
5) Y. Mizuno and T. Sasaki, *Tetrahedron Letters*, **1965**, 4579.
6) U. Brodbeck and J.G. Moffatt, *J. Org. Chem.*, **35**, 3552 (1970).
7) H-U. Blank and W. Pfleiderer, *Ann. Chem.*, **742**, 16 (1970).
8) N.C. Yang and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961).
9) J. Zemlicka, *Coll. Czech. Chem. Commn.*, **29**, 4464 (1964).

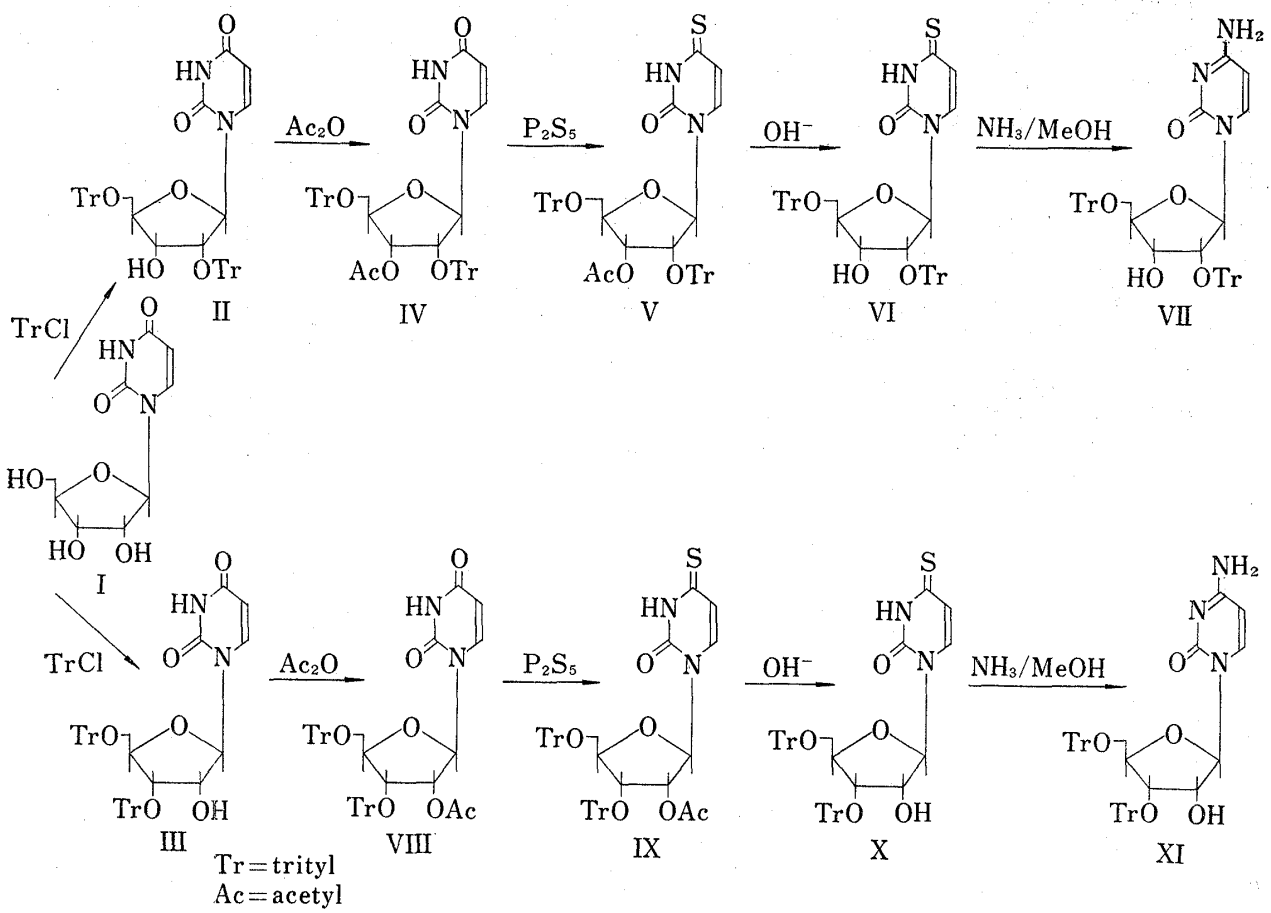

Chart 1

In the similar manner, 3′,5′-di-O-tritylcytidine (XI) was prepared by thiation of 3′,5′-di-O-trityl-2′-O-acetyluridine (VIII), followed by amination of the 4-thio derivative, IX, as illustrated in Chart 1. While the product (XI) was reconverted to the starting material, 3′,5′-di-O-trityluridine (III), by treatment with *n*-amyl nitrite, the treatment with 98% formic acid at room temperature readily gave cytidine, confirming its identity as 3′,5′-di-O-tritylcytidine (XI).

It is noted that di-O-tritylated uridine easily undergoes the reaction with phosphorus pentasulfide in refluxing toluene containing pyridine to give the 4-thio derivatives without being detritylated. When toluene or pyridine alone was used as the solvent, the yield was remarkably decreased as shown in Table I.

Table I. Thiation of 2′,5′-Di-O-trityl-3′-O-acetyluridine (IV)

Toluene (%)	Pyridine (%)	P_2S_5	Yield of V (%)	IV
100	0	2 equ.	23	+
90	10	2 equ.	49	+
80	20	2 equ.	83	−
70	30	2 equ.	77	−
60	40	2 equ.	56	+
50	50	2 equ.	45	+
40	60	2 equ.	37	+
30	70	2 equ.	21	+
20	80	2 equ.	23	+
10	90	2 equ.	19	+
0	100	2 equ.	20	+

2′,5′-Di-O-trityl-3′-O-methanesulfonyluridine[8] (XII) was also thiated in a similar manner to the corresponding 4-thio derivative in good yield. This compound (XIII) was converted to the cytidine derivative (XIV) by treatment with methanolic ammonia. The product (XIV) was identical with 2′,5′-di-O-trityl-3′-O-methanesulfonylcytidine obtained from the reaction of VII with an equivalent amount of methanesulfonyl chloride, by mixed melting point and infrared (IR) spectra. Compound XIII was converted to 1-(β-D-2′,5′-di-O-tritylxylofuranosyl)cytosine[6] XV *via* unstable 2,3′-anhydro intermediate[5] by the treatment with potassium *t*-butoxide in dimethylformamide.

Phosphorylation of VII and XI was carried out by Tener's method.[10] After the removal of the protecting groups, the resulting phosphate ester was analysed by Dowex 1 column chromatography, which indicated less than 5% of phosphate migration in each case.

A selective N-acetylation of VII with an equivalent acetic anhydride in refluxing pyridine[11] gave 2′,5′-di-O-trityl-N⁴-acetylcytidine.[5] (XVI) Then, XVI was allowed to react with 2′,3′-di-O-acetyluridine 5′-phosphate[12] using triisopropyl benzenesulfonyl chloride

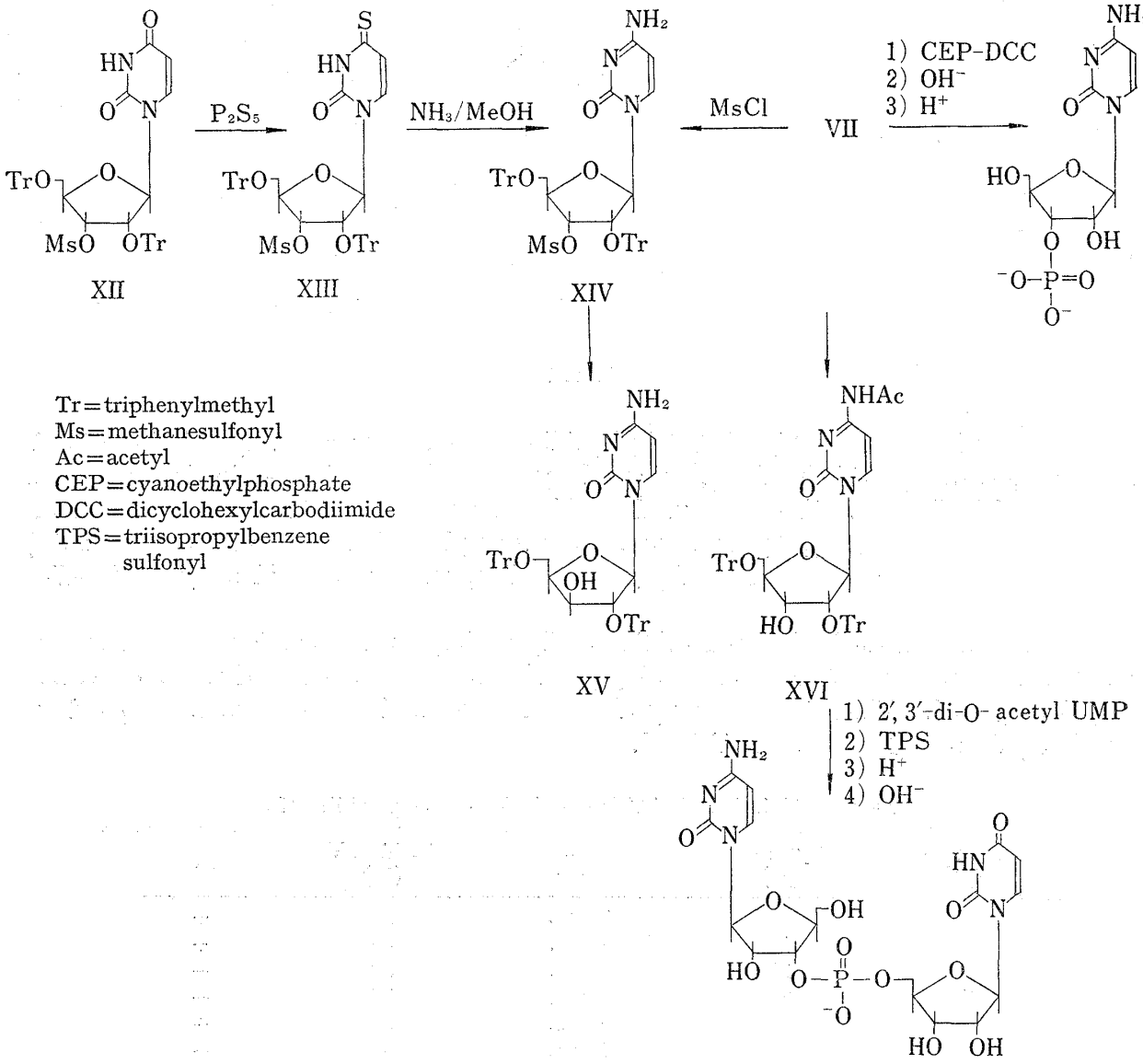

Chart 2

10) G.M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
11) T. Sasaki and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **15**, 894 (1967).
12) M. Saneyoshi, *Chem. Pharm. Bull.* (Tokyo), **19**, 493 (1971).

as a condensing agent. After successive treatments of the resulting blocked dinucleoside monophosphate with formic acid and with methanolic ammonia, cytidylyl (3′→5′) uridine (XVII) was obtained in 50% yield. It was characterized by degradation to cytidine 3′-phosphate and uridine (1:1) with pancreatic ribonuclease and also with snake venom phosphodiesterase to cytidine and uridine 5′-phosphate (1:1). From these results, di-O-tritylcytidines and di-O-trityl-4-thiouridine derivatives, such as V, VI, VII, IX, X and XI may be used as the key intermediates for synthesis of other 4-substituted di-O-trityl pyrimidine ribonucleosides, sugar-modified nucleosides and oligonucleotides.

Experimental

2′,5′-Di-O-trityl-3′-O-acetyluridine (III)——To a solution of 13 g of 2′,5′-di-O-trityluridine (II) in 120 ml of anhydrous pyridine, 26 ml of freshly distilled acetic anhydride was added. The clear mixture was stored at room temperature in the dark for 24 hr under protection from moisture. The solvent and excess acetic anhydride were removed under reduced pressure and the residual gum was heated in 60 ml of refluxing methanol for 15 min. After removal of solvent, the acid-free gum was crystallized from benzene–cyclohexane to give fine white crystals mp 148—150° (13 g, 97%). UV λ_{max}^{EtOH} nm: 218, 261. *Anal.* Calcd. for $C_{49}H_{42}O_7N_2$: C, 76.36; H, 5.45; N, 3.63. Found: C, 76.42; H, 5.41; N, 3.59.

2′,5′-Di-O-trityl-3′-O-acetyl-4-thiouridine (V)——Five grams of IV and 4.8 g of freshly ground phosphorus pentasulfide was refluxed in a mixture of anhydrous toluene (70 ml) and pyridine (30 ml) for 3 hr. The reaction mixture was allowed to cool to room temperature, the dark yellow supernatant liquid was decanted and the residue was washed with hot benzene (10 ml×3). The supernatant and washings were combined and concentrated to dryness. The residual gum was dissolved in 40 ml of a mixture of chloroform and water (1:1) and was vigorously shaken for 30 min. The chloroform layer was dried over magnesium sulfate and concentrated to a small volume (*ca.* 5 ml). This was chromatographed on silica gel (3 cm×40 cm) column by using chloroform as an eluent. Crystallization from benzene–cyclohexane gave 3.9 g (78%) of yellow fine prisms. mp 158—160°. UV λ_{max}^{EtOH} nm: 244, 329. *Anal.* Calcd. for $C_{49}H_{42}O_6N_2S$: C, 74.80; H, 5.34; N, 3.56. Found: C, 74.75; H, 5.30; N, 3.53.

2′,5′-Di-O-trityl-4-thiouridine (VI)——A solution of 300 mg of V was refluxed in 35 ml of 0.5N sodium methoxide in dry methanol for 3 hr. After evaporation of the solvent, the residue was dissolved in 30 ml of chloroform and washed with water. The organic layer separated and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residual gum was crystallized from benzene–cyclohexane to give 248 mg (85%) of yellow micro-needles. mp 223—225°. UV λ_{max}^{EtOH} nm: 245, 330. *Anal.* Calcd. for $C_{47}H_{40}O_5N_2S$: C, 75.80; H, 5.38; N, 3.76. Found: C, 75.87; H, 5.33; N, 3.81.

2′,5′-Di-O-tritylcytidine (VII)——Method A: A solution of 1 g of V in 50 ml of methanol saturated with ammonia was allowed to stand at 0° in a sealed tube and then heated at 100° for 10 hr. The reaction mixture was concentrated to dryness under reduced pressure. A gummy material was crystallized from ethanol to give white leaflets. 790 mg (85%); mp 181—182° (lit. 178—180°,[5] 180—182°,[6] 179°[7]). UV λ_{max}^{EtOH} nm: 270 (ε=8100). *Anal.* Calcd. for $C_{47}H_{41}O_5N_3$: C, 77.54; H, 5.68; N, 5.75. Found: C, 77.49; H, 5.59; N, 5.71.

Method B: In a similar VI as a starting material gave same product which was identical with 2′,5′-di-O-tritylcytidine in 87% yield.

Conversion of VII to 2′,5′-Di-O-trityluridine (II) by the Treatment with *n*-Amyl Nitrite——Compound VII (50 mg) was dissolved in a mixture of 3 ml of dimethyl sulfoxide and 0.5 ml of acetic acid. One ml of *n*-amyl nitrite was added dropwise under vigorous stirring at 0°. After continuous stirring for 2.5 hr, the reaction mixture was poured into a mixture of 30 ml of ice-water and 30 ml of ether. After shaking the mixture for 10 min, precipitates were collected by filtration. It was washed with ether, and was dried. Crystallization from benzene–ether gave microneedles, 32 mg (65%); mp 225—226°. The product was identical with authentic 2′,5′-di-O-trityluridine by measurement of the mixed melting point, ultraviolet (UV) and infrared (IR) spectra, and chromatographic behavior on silica gel.

Detritylation of VII with Formic Acid to Cytidine——Compound VII (50 mg) was suspended in 50 ml of 98% formic acid and stirred at room temperature for 30 min. The suspension became clear and then white precipitates of trityl alcohol appeared. After stirring for 1.5 hr, the precipitates were removed by filtration and washed with 50% formic acid (3 ml). The filtrate and washings were combined and evaporated under reduced pressure at room temperature. The residue was dissolved in 3 ml of distilled water and then lyophilized. This sample was identical with cytidine according to paper chromatographic mobilities in several solvent systems and UV and IR spectrophotometric measurements.

3′,5′-Di-O-trityl-2′-O-acetyluridine (VIII)——To a solution of III (5 g) in 60 ml of anhydrous pyridine 10 ml of freshly distilled acetic anhydride was added. It was allowed to stand at room temperature for 24 hr. After evaporation of solvent, the product was crystallized from methanol to give 5 g (97%) of VIII.

mp 108—110°. *Anal.* Calcd. for $C_{49}H_{42}O_7N_2$: C, 76.36; H, 5.45; N, 3.63. Found: C, 76.44; H, 5.49; N, 3.66.

3′,5′-Di-O-trityl-2′-O-acetyl-4-thiouridine (IX)——A mixture of VIII (5 g) and phosphorus pentasulfide (4.6 g) in toluene (150 ml) containing dry pyridine (50 ml) was refluxed for 4 hr. Compound IX was isolated by using silica gel column (1.5×50 cm) chromatography eluted. It was crystallized from benzene–cyclohexane to afford yellow crystals. Yield: 4.6 g (92%). mp 117—119°. UV λ_{max}^{EtOH} nm: 245, 331. *Anal.* Calcd. for $C_{49}H_{42}O_6N_2S$: C, 74.80; H, 5.34; N, 3.56. Found: C, 74.75; H, 5.31; N, 3.52.

3′,5′-Di-O-trityl-4-thiouridine (X)——A solution of IX (200 mg) in 50 ml of 0.5N methanolic sodium methoxide was gently refluxed for 3 hr. After evaporation of the solvent, the residue was dissolved in 30 ml of chloroform and washed with water. The organic layer separated and dried over $MgSO_4$. After removal of solvent under reduced pressure, crystallization from benzene–cyclohexane gave X. Yield: 155 mg (80%). mp 176—177°. *Anal.* Calcd. for $C_{47}H_{40}O_5N_2S$: C, 75.80; H, 5.38; N, 3.76. Found: C, 75.76; H, 5.40; N, 3.69.

3′,5′-Di-O-tritylcytidine (XI) from IX——A solution of IX (50 mg) in 5 ml of dry methanol saturated with ammonia at 0°, which was heated in a sealed tube at 100° for 10 hr. The reaction mixture was concentrated to dryness under reduced pressure. Crystallization of the residue from ethanol gave 3′,5′-di-O-tritylcytidine (XI). Yield: 35 mg (75%). mp 222—224° (lit.[6] 225—226°). UV λ_{max}^{MeOH} nm: 270 (ε=8600). *Anal.* Calcd. for $C_{47}H_{41}O_5N_3$: C, 77.54; H, 5.68; N, 5.78. Found: C, 77.49; H, 5.62; N, 5.77.

Conversion of XI to III——Compound XI (15 mg) was treated with *n*-amyl nitrite (0.5 ml) in 1.5 ml of dimethylsulfoxide in the presence of acetic acid (0.1 ml) to give III. 12 mg (82%). This was identical with III prepared earlier[9] according to mixed melting point test and measurement of IR spectra.

2′,5′-Di-O-trityl-3′-O-methanesulfonyl-4-thiouridine (XIII)——To a solution of 2′,5′-di-O-trityl-3′-O-methanesulfonyluridine[8] (XII) (3 g) in a mixture of 70 ml of toluene and 30 ml of pyridine was added 3.5 g of freshly ground phosphorus pentasulfide. The mixture was refluxed for 3 hr. After removal of solvent, the residue was dissolved in 10 ml of 50% aqueous ethanol and it was concentrated. This process of addition and evaporation of ethanol repeated until the odor of pyridine and hydrogen sulfide was no longer evident. The gummy solid was dissolved in small amount of chloroform (*ca.* 3 ml) and applied to the column of silica gel (3×40 cm). Elution was performed with a mixture of chloroform and ethyl acetate (9: 1). Light yellow crystalline was obtained. Recrystallization from a mixture of chloroform and cyclohexane gave fine yellow needles. Yield: 3 g (94%). mp 206—208°. UV λ_{max}^{EtOH} nm: 245, 331. IR ν_{max}^{KBr}: 1170 cm^{-1} (sulfonate).

2′,5′-Di-O-trityl-3′-O-methanesulfonylcytidine (XIV)——Method A: To a solution of 200 mg of VII in 10 ml of anhydrous pyridine cooled in an ice-bath, 1.2 equivalents of freshly distilled methanesulfonyl chloride was added dropwise. The mixture was allowed to stand at 0° overnight. After addition of 0.5 ml of water, the mixture was stored in an ice-box for 1 hr. Then it was poured into 30 ml of ice-water with vigorous stirring. The granular product was collected by filtration and washed with water and dried in air. Recrystallization from ethanol gave white fine needles, 200 mg (90%). mp 185—187° (lit. 180—183°[5]). UV λ_{max}^{EtOH} nm: 270. IR ν_{max}^{KBr}: 1172 cm^{-1} (sulfonate). *Anal.* Calcd. for $C_{48}H_{43}O_7N_3S$: C, 71.55; H, 5.34; N, 5.34. Found: C, 71.70; H, 5.26; N, 5.47.

Method B: 2′,5′-Di-O-trityl-3′-O-methanesulfonyl-4-thiouridine (1 g) in 30 ml of methanol saturated with ammonia was sealed in a pyrex tube and the mixture was heated at 100—110° for 10 hr. The volatiles were removed under reduced pressure and the residue was crystallized from ethanol to give white needles, 890 mg. This was identical to a sample prepared by "Method A."

Selective N-Acetylation of VII——Acetic anhydride (44 mg) was added to a solution of VII (360 mg) in dry pyridine (30 ml) and the mixture was refluxed for 3 hr. After cooling, the solvent was removed under reduced pressure. The residue was treated with 10 ml of 50% aqueous ethanol and then the solvent was evaporated. This was repeated three times. The resulting gum was crystallized from ethanol to give needles. 325 mg (85%). Recrystallization from ethanol, mp 175—179° (lit. 168—170°,[5] lit. 170—180°[6]). UV λ_{max}^{EtOH} nm: 305, 250 (shoulder). IR ν_{max}^{KBr}: 1710 cm^{-1}. *Anal.* Calcd. for $C_{49}H_{43}O_6N_3$: C, 76.43; H, 5.63; N, 5.46. Found: C, 76.15; H, 5.43; N, 5.38.

Phosphorylation of 2′,5′- and 3′,5′-Di-O-tritylcytidines——To a solution of pyridinium β-cyanoethylphosphate (1 mmole) in 30 ml of anhydrous pyridine, 850 mg (4 mmole) of dicyclohexylcarbodiimide was added at once and then 0.5 mmole of 2′,5′- or 3′,5′-di-O-tritylcytidine was added. The reaction flask was stoppered and allowed to stand at room temperature for 48 hr. The mixture was treated with 3 ml of water and kept an additional hour at room temperature. After removal of the precipitate by filtration, the solvent was evaporated. The solid residue was suspended in 5 ml of 1N lithium hydroxide and it was heated in a boiling water-bath for 15 min. The reaction mixture was neutralized with Dowex-50 (H^+ form) resin and the resin was filtered. The filter cake was extracted with 5 portions of hot ethanol (30 ml). Evaporation of the extract gave a gum. This was dissolved in 10 ml of 98% formic acid and it was stirred at room temperature for 2 hr. Precipitate appeared and was removed by filtration and the filtrate was lyophilized. Paper chromatography showed only one spot (Rf=0.47) in the solvent system of isobutylic acid–0.5N ammonium hydroxide (5: 3, v/v, pH 3.8). Then *ca.* 50 O.D. A_{275} units of the product was applied to Dowex 1 (formate form) column using 0.05N formic acid as an eluting solvent. The elution prophile showed that only small amount (*ca.* 5%) of phosphate migration had taken place in each case.

1-(2′,5′-Di-O-trityl-β-D-xylofuranosyl)-cytosine (XV)——2′,5′-Di-O-trityl-3′-methanesulfonylcytidine (100 mg) was treated with potassium *t*-butoxide (1.5 equivalent) in 30 ml of dry dimethylformamide at 100° for 3 hr. The reaction mixture was poured into ice-water and precipitates were collected, washed with water and dried. Recrystallization from ethanol gave fine crystal (75 mg) which melted at 175—179°, resolidified and remelted at 235—240° (lit.[6] 178—180° and 250°). UV λ_{max}^{EtOH} nm: 267.5. No sulfonate signal on IR spectrum.

Synthesis of Cytidylyl-(3′→5′)-uridine (XVII)——A mixture of XVI (1 mmole) and pyridinium 2′,3′-di-O-acetyluridine 5′-phosphate[12] (1.1 mmole) was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (450 mg, 1.5 mmole) in 50 ml of anhydrous pyridine. The resulting solution was kept in a tightly stoppered flask in the dark at room temperature for 15 hr. The reaction mixture was then treated with 10 ml of water and kept at room temperature for 3 hr. After removal of solvent under reduced pressure, the residue was dissolved in 50 ml of formic acid. The solution was stirred at room temperature for 3 hr. Precipitate was removed by filtration. The filtrate was concentrated and the residue was extracted with ether (10 ml × 5). The residue was treated with methanolic ammonia (50 ml) and it was allowed to stand at room temperature for 18 hr. The solvent was removed under reduced pressure. The residue was dissolved in 5 ml of water. On paper chromatogram three spots were detected, namely, cytidine (Rf=0.75), cytidylyluridine (Rf=0.38) and uridine 5′-phosphate (Rf=0.20). The product was applied to the column of DEAE-cellulose (bicarbonate form) (2 × 40 cm) and eluted by gradient elution with 0.1M triethylammonium bicarbonate (pH 7.8) (500 ml) and 500 ml of water in the mixing chamber. The fractions of second peak were combined and concentrated. The product was homogeneous on paper chromatogram (Rf=0.38), 0.5 mmole (50%).

Digestion of Cytidylyluridine with Pancreatic Ribonuclease and Snake Venom Phosphodiesterase——The structure of the cytidylyl-(3′-5′-)-uridine prepared in the above experiment was determined by enzymatic digestion in the presence of pancreatic RNase or snake venom phosphodiesterase. The degradation products were separated by paper chromatography. Paper chromatography was performed using Whatman No. 3MM paper. Solvent system used was isobutylic acid–0.5N ammonium hydroxide (5: 3 v/v, pH 3.8). Estimation of the compounds from the individual spots was carried out spectrophotometrically after elution from paper with 0.01N hydrochloric acid. The results of the enzymatic degradation are summarized as follows.

Substrate	Enzyme	Digestion yield	Ratio of product
CpU	pancreatic RNase	95%	cytidine 3′-phosphate 0.97 : uridine 1
CpU	snake venom phosphodiesterase	100%	cytidine 1 : uridine 5′-phosphate 1.05

Acknowledgement The author is indebted to Dr. Y. Kawazoe of this institute and Dr. G.M. Tener of the Department of Biochemistry, The University of British Columbia, for their critically reading this manuscript. Thanks are also due to Mr. K. Takahashi for his assistance in the preparation of 2′,5′- and 3′,5′-di-O-trityluridine.