

OLIGONUCLEOTIDIC COMPOUNDS. XLIV.*

PROTECTION OF THE INTERNUCLEOTIDIC BOND AFTER
ITS SYNTHESIS AS APPROACH TO THE SYNTHESIS
OF AN OLIGONUCLEOTIDIC CHAIN**

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Received March 29th, 1973

Reaction of 5'-O-dimethoxytrityl-2'-O-tetrahydropyranylluridine 3'-phosphate (*Ia*) with 2',3'-di-O-benzoyluridine in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride and the subsequent treatment with 3-hydroxypropionitrile affords 5'-O-dimethoxytrityl-2'-O-tetrahydropyranylluridylyl-(3'→5')-2',3'-di-O-benzoyluridine [P¹-(2-cyanoethyl) ester] (*Iia*). Removal of the dimethoxytrityl group and repetition of the reaction with compound *Ia* and 3-hydroxypropionitrile leads to the P¹,P²-bis(2-cyanoethyl) ester of the trinucleoside diphosphate *V*. The P¹,P²,P³-tris(2-cyanoethyl) ester of the tetranucleoside triphosphate *XI* is obtained analogously from 5'-O-dimethoxytrityl-2'-O-acetyluridine 3'-phosphate (*Ic*) by a stepwise synthesis of the internucleotidic bond and its protection.

In earlier papers^{2,3}, some aspects of the triester synthesis of the internucleotidic bond in the ribo series have been investigated. The approach *via* the 2,2,2-trichloroethyl group in the role of the protecting group of the internucleotidic bond was abandoned because of the low yields in the final deblocking step. In hands of Neilson and Werstiuk, however, the same 2,2,2-trichloroethyl group proved very suitable for the triester synthesis in the ribo series, since excellent yields were obtained in all reaction steps^{4,5}. In continuation of our investigations, a procedure has been now attempted starting from 5'-O-dimethoxytrityl derivatives of ribonucleoside 3'-(2-cyanoethyl) phosphates, protected at the 2'-hydroxylic function with a tetrahydropyranyl or acetyl group, because of the ready accessibility of the specifically substituted ribonucleoside 3'-phosphates (for references see papers^{2,3}). Furthermore, the use of the dimethoxytrityl group proved advantageous for the identification of intermediates by a color test with perchloric acid⁶ in separations of the reaction mixture by preparative thin-layer chromatography; detection under ultraviolet light is not possible because of the simultaneous presence of dark-coloured by-products and pyridine.

* Part XLIII: This Journal 38, 2962 (1973).

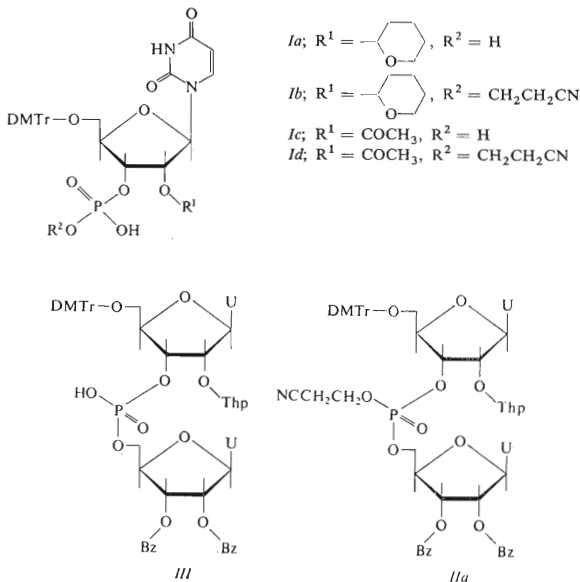
** Parts of this work have been reported in a preliminary communication¹.

As the starting material for the stepwise triester synthesis, 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyluridine 3'-(2-cyanoethyl) phosphate (*Ib*) and the analogous 2'-O-acetyl derivative *Id* were used. Compound *Ib* was prepared from 5'-O-acetyl-2'-O-tetrahydropyranyl 3'-phosphate⁷ by the successive deacetylation, dimethoxytritylation, and treatment with 3-hydroxypropionitrile in the presence of N,N'-dicyclohexylcarbodiimide. When the last step of this preparation is performed according to the original procedure⁸, there are formed considerable amounts of the bis(2-cyanoethyl) ester which may be converted in the case of the 2'-O-tetrahydropyranyl derivative in weakly alkaline media to the required compound *Ib*. In the preparation of the analogous 2'-O-acetyl derivative *Id*, however, the alkaline treatment is not possible because of the high lability of the 2'-O-acetyl group in the neighbourhood of 3'-phosphate. The original esterification procedure of the monophosphate with 3-hydroxypropionitrile was modified by replacement of the pyridinium salt of the phosphate by the monotriethylammonium salt. The modified procedure affords the nucleoside 3'-(2-cyanoethyl) phosphate as the single product.

The triester syntheses starting from compounds *Ib* and *Id* were in both cases performed with the use of three equivalents of 2',3'-di-O-benzoyluridine and two equivalents of 2,3,5-triisopropylbenzenesulfonyl chloride. The reaction mixtures were separated by preparative chromatography on loose layers of silica gel. When pyridine was removed from the reaction mixture prior to the isolation step, the chromatography was accompanied to a considerable extent by cleavage of the dimethoxytrityl group. With the use of the solvent systems chloroform-methanol-pyridine, however, the detritylation did not occur and the separation improved. The yields of the first step (one equivalent of the phosphate component per two equivalents of the hydroxylic component) varied between 65–70%.

In the subsequent step of the trinucleoside diphosphate derivative synthesis, the dimethoxytrityl group was removed on treatment with 90% aqueous acetic acid. In the series of 2'-O-acetyl derivatives, this step is not connected with any problems. On the other hand in the series of 2'-O-tetrahydropyranyl derivatives, the detritylation may be accompanied by a simultaneous removal of the tetrahydropyranyl group. As observed by Smith and coworkers⁹ in investigations on the removal of the dimethoxytrityl group from 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyluridine 3'-phosphate, a high selectivity of the detritylation can be obtained when the reaction is performed with 80% aqueous acetic acid at 0°C. In the latter case, the unfavourable labilisation effect of the C_(3')-phosphoryl group must be taken into account. When the C_(3')-phosphoryl group is esterified, its labilisation effect on the 2'-O-tetrahydropyranyl group is negligible and the removal of the dimethoxytrityl group at 0°C is consequently selective, as observed in this Laboratory³. It has been now found with the triester *IIIa*, that in accordance with ref.³ the removal of the dimethoxytrityl group is quite selective.

The trinucleoside diphosphate derivative step of the triester synthesis consisted



SCHEME 1

in the simultaneous action of two equivalents of the phosphodiester *Ib* or *Id* and four equivalents of 2,3,5-triisopropylbenzenesulfonyl chloride on one equivalent of dinucleoside 2-cyanoethyl esters *IIB* or *VIII* (carrying a free hydroxylic function). After 20 h of the reaction, the yields were about 35%. The yields did not considerably increase when longer reaction periods of time (3 days) were used. Some by-products were also isolated, probably diribonucleoside phosphate derivatives arising by reaction of the sulfonyl chloride with the $C_{(3,5)}$ -hydroxylic functions of compounds *IIB* or *VIII*. The formation of these sulfonyl derivatives is in accordance with observations of Lohrman and Khorana¹⁰ on the 9% sulfonylation of 2',3'-di-O-benzoyluridine on treatment with the same sulfonyl chloride for 20 h.

In view of the poorly satisfactory yields of the triester synthesis at the early trinucleoside diphosphate stage, the use of this route for the synthesis of longer chains did not appear promising. We have therefore reconsidered the approach^{2,3}

chloride (10 equivalents; after several hours, 3-hydroxypropionitrile (20 equivalents) was added. After additional 15–20 h, the crude dimethoxytrityl derivatives *IV* and *IX*, resp., along with the bis(2-cyanoethyl) esters of the starting nucleotides, were isolated by the preparative thin-layer chromatography and subjected to detriptylation. The final chromatography afforded pure products *V* and *X* in 68 and 65% yields, resp. In the series of 2'-O-acetyl derivatives, an additional chain-lengthening was carried out under the formation of the tetranucleoside triphosphate *XII*; overall yield, 46%. For the abbreviations in Scheme 2 see ref.¹²

The reported "combined" synthesis of the oligonucleotidic chain affords considerably higher yields than the triester synthesis from nucleotide 2-cyanoethyl esters.

EXPERIMENTAL

Thin-layer chromatography as well as the preparative runs were performed similarly to the preceding paper¹¹ in the solvent systems T_1 , 2-propanol-concd. aqueous ammonia-water (7 : 1 : 2); T_2 , chloroform-methanol (9 : 1); T_3 , chloroform-methanol-pyridine (94 : 1 : 5); T_4 , chloroform-methanol-pyridine (8 : 1 : 1); T_5 , chloroform-methanol (85 : 15); T_6 , chloroform-methanol-pyridine (70 : 15 : 15); and T_7 , chloroform-methanol (8 : 2). Bands were eluted with the solvent system T_6 , chloroform-methanol (1 : 1). For the paper electrophoresis see ref.¹¹ The coupling mixtures were taken down on a rotary evaporator at 20°C/1 Torr with the use of a dry-ice-condenser; the ordinary pressure was restored through a silica gel column.

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyluridine 3'-Phosphate (*Ia*)

A mixture of the calcium salt of 5'-O-acetyl-2'-O-tetrahydropyranyluridine 3'-phosphate⁷ (12.6 g), 50% aq. pyridine (40 ml), pyridinium Dowex 50 (40 ml), and concd. NH_4OH (60 ml) is stirred for 15 h. Pyridine (50 ml) is then added and the ammonia (about 100 ml of the distillate) is evaporated at 35°C/15 Torr. The residual mixture is applied to a column (500 ml) of pyridinium Dowex 50 ion exchange resin and the column is eluted with 50% aqueous pyridine (700 ml). The effluent is evaporated, the residue coevaporated with three portions of pyridine, and finally dissolved in pyridine (100 ml). Dimethoxytrityl chloride (15 g) is then added and the mixture is stirred to afford a solution which is kept at room temperature for 20 h. Triethylamine (10 ml) in ethanol (50 ml) is slowly added under cooling with water, the mixture kept for 15 min, and treated with water (50 ml). After additional 15 min the reaction mixture is extracted with ether (200 ml) and the ethereal extract is washed with water (50 ml). Pyridine (20 ml) and sodium chloride (2 g) is then added to the aqueous layer and the whole is extracted with two 200 ml portions of chloroform. The chloroform extracts are dried over magnesium sulfate, treated with triethylamine (5 ml), evaporated, and the residue is coevaporated with two portions of pyridine. The residue is dissolved in pyridine (50 ml) and the solution added dropwise into ether (1500 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure to afford 12.8 g of the triethylammonium salt of compound *Ia*. Molecular weight 785, as determined spectrophotometrically from the spot eluate of uridylic acid after deblocking the aliquot with 80% aqueous acetic acid and chromatography on paper Whatman No 1 in the solvent system T_1 . For $\text{C}_{35}\text{H}_{39}\text{N}_2\text{O}_{12}\text{P}\cdot\text{C}_6\text{H}_{15}\text{N}$ (811.8) calculated: 5.18% N, 3.82% P; found: 5.30% N, 3.66% P. R_F value in T_1 , 0.32.

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyluridine 3'-(2-Cyanoethyl) Phosphate (*Ib*)

A solution of the triethylammonium salt of compound *Ia* (13.7 g) in 50% aqueous pyridine (50 ml) is passed through a column (60 ml) of pyridinium Dowex 50 ion exchange resin and the column is eluted with 50% aqueous pyridine (200 ml). The eluates are evaporated, the residue coevaporated six times with pyridine, and finally dissolved in pyridine (50 ml). N,N'-Dicyclohexylcarbodiimide (15 g), 3-hydroxypropionitrile (15 ml), and triethylamine (2.1 ml) are then added and the whole kept at room temperature for 20 h. The mixture is shaken with water (50 ml) and cyclohexane (100 ml) and filtered. Sodium chloride is added (2 g) to the lower layer of the filtrate and the mixture is extracted with chloroform. The extract is dried over magnesium sulfate and evaporated at 35°C/15 Torr under the occasional addition of pyridine. The residual sirup is triturated with ether and the ethereal washings are decanted. The residual substance is dissolved in pyridine (100 ml) and the solution is added dropwise under stirring into ether (2000 ml). The precipitate is collected with suction, washed with ether, and dried under diminished pressure to afford 12.3 g of the triethylammonium salt of compound *Ib*. For $C_{38}H_{42}N_3O_{12}P \cdot C_6H_{15}N$ (864.9) calculated: 6.47% N, 3.58% P; found: 6.02% N, 3.12% P. R_F value in T_1 , 0.46.

5'-O-Dimethoxytrityl-2'-O-acetyluridine 3'-Phosphate (*Ic*)

The title compound was prepared according to the ref.¹³ except for the pancreatic ribonuclease degradation of 5'-O-dimethoxytrityluridine 2',3'-cyclic phosphate which was performed in 30% aqueous dimethylformamide buffered by 0.2M triethylammonium hydrogen carbonate (pH 7.5).

5'-O-Dimethoxytrityl-2'-O-acetyluridine 3'-(2-Cyanoethyl)phosphate (*Id*)

The pyridinium salt of compound *Ic* (4.5 g) is coevaporated with two portions of pyridine and the residue is dissolved in pyridine (10 ml). N,N'-Dicyclohexylcarbodiimide (4 g), 3-hydroxypropionitrile (4 ml), and triethylamine (0.56 ml) are then added to the solution and the whole is kept at room temperature for 4 days. The mixture is shaken with water (8 ml) and cyclohexane (20 ml), filtered, and the lower layer of the filtrate extracted with ethyl acetate (100 ml). The extract is washed with 25% aqueous pyridine (100 ml) and water (100 ml), dried over magnesium sulfate, and evaporated. The residue is washed by trituration with ether (200 ml), dissolved in pyridine (10 ml), and the solution is added dropwise under stirring into ether (200 ml). The precipitate is collected by centrifugation, washed with ether, and dried under diminished pressure to afford 4.3 g of the triethylammonium salt of compound *Id*. For $C_{35}H_{36}N_3O_{12}P \cdot C_6H_{15}N$ (822.8) calculated: 6.81% N, 3.77% P; found: 6.28% N, 3.41% P.

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyluridylyl-(3'→5')-2',3'-di-O-benzoyluridine P-(2-Cyanoethyl) Ester (*Ila*)

A. Triester synthesis. A mixture of the triethylammonium salt of the 2-cyanoethyl ester *Ib* (2 mmol) and 2',3'-di-O-benzoyluridine (2.8 g; 6 mmol) is coevaporated at 20°C/1 Torr with three portions of pyridine, the residue is shaken for several minutes with 2,3,5-triisopropylbenzenesulfonyl chloride (1.8 g) and pyridine (10 ml), and evaporated just to the beginning of crystallisation. The concentrate is stored in a desiccator for 2 days, diluted with chloroform (5 ml), and chromatographed on four 20×20×0.6 cm layers of loose silica gel in the solvent system T_3 . The dimethoxytrityl-group-positive bands (R_F value from 0.55 to 0.74) were eluted with T_c , the eluates coevaporated with toluene, and the crude residue rechromatographed on three 40×16×0.6 cm layers of loose silica gel in T_2 . The ultraviolet-absorbing bands (R_F , 0.50) were

eluted and the eluates evaporated to afford 1.69 g (70%) of compound *Ila* in the form of a foam (R_F value in T_2 , 0.72). The characterisation was performed on treatment with dilute aqueous ammonia (5 min at 20°C) to afford quantitatively the diester *III* (R_F in T_2 : 0.72 → 0.05).

B. Combined synthesis. A mixture of the triethylammonium salt of the nucleotide *Ia* (0.5 mmol) and 2',3'-di-O-benzoyluridine (452 mg; 1 mmol) is coevaporated with three portions of pyridine, the residue is shaken for several minutes with 2,3,5-triisopropylbenzenesulfonyl chloride (750 mg) and pyridine (5 ml), and evaporated just to the beginning of crystallisation. The concentrate is kept at room temperature for 6 h, treated with 3-hydroxypropionitrile (0.33 ml), kept for additional 20 h, and processed similarly to procedure *A*. Yield, 545 mg (90%) of compound *Ila*.

2'-O-Tetrahydropyranlyrididylyl-(3'→5')-2',3'-di-O-benzoyluridine (P-(2-Cyanoethyl) Ester (*Ib*))

A solution of compound *Ila* (1.55 g) in 90% aqueous acetic acid (25 ml) is kept at 0°C for 16 h, evaporated at 20°C/1 Torr, and the residue coevaporated with two portions of 1-butanol. The final residue is dissolved in chloroform and chromatographed on three 40×16×0.6 cm layers of loose silica gel in the solvent system T_2 . Elution and evaporation of the ultraviolet-absorbing bands (R_F , 0.39) afforded 960 mg (83%) of a solid foam of compound *Ib* which forms a double spot of diastereoisomers (R_F values, 0.28 and 0.31) when chromatographed on a thin layer of silica gel in T_2 . The analytical sample was rechromatographed under the above conditions. For $C_{40}H_{42}N_5O_{17}P$ (895.6) calculated: 7.81% N, 3.45% P; found: 7.62% N, 3.28% P. When treated with dilute aqueous ammonia at 50°C for 1 h, compound *Ib* affords 2'-O-tetrahydropyranlyrididylyl-(3'→5')-uridine which is not degraded by pancreatic ribonuclease.

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranlyrididylyl-(3'→5')-2'-O-tetrahydropyranlyrididylyl-(3'→5')-2',3'-di-O-benzoyluridine P¹,P²-Bis(2-cyanoethyl) Ester (*IV*)

The 2-cyanoethyl ester *Ib* (0.25 mmol) is evaporated three times with pyridine and the residue is shaken with 2,3,5-triisopropylbenzenesulfonyl chloride (160 mg) and pyridine (3 ml) for 30 min. The triester *Iic* (0.12 mmol) in pyridine (5 ml) is then added, the whole mixture is evaporated to dryness, the residue is redissolved in pyridine (10 ml), the solution is concentrated just to the beginning of crystallisation, the concentrate kept at room temperature for 2 days, diluted with chloroform (3 ml), and chromatographed on a 20×20×0.6 cm layer of loose silica gel in the solvent system T_4 to afford two dimethoxytrityl-group-positive bands, R_F values 0.43 and 0.60 to 0.85. The less mobile band was eluted, the eluate coevaporated with toluene, and the residue rechromatographed on a 40×16×0.6 cm layer of silica gel in T_2 to afford (from the R_F 0.53 band) 72 mg (36%) of compound *IV* (R_F in T_2 , 0.52) and (from the R_F 0.39 band) 25 mg (23%) of the starting compound *Ib*. The faster band from the first chromatography afforded after complete deblocking (in addition to Up) as the principal product a substance, the electrophoretical (E_{Up} , 0.50) and chromatographical (R_F in T_1 , 0.40) properties of which suggest the structure of a sulfonyl derivative of uridylyl-uridine. The product *IV* was characterised by a quantitative conversion into UpUpU, on successive deblocking.

2'-O-Tetrahydropyranlyrididylyl-(3'→5')-2'-O-tetrahydropyranlyrididylyl-(3'→5')-2',3'-di-O-benzoyluridine P¹,P²-Bis(2-cyanoethyl) Ester (*V*)

A. A solution of compound *IV* (170 mg) in 90% aqueous acetic acid (5 ml) is kept at 0°C for 15 h, evaporated at 10°C/1 Torr, the residue coevaporated twice with 1-butanol, and finally chromatographed on a 40×16×0.6 cm layer of loose silica gel in the solvent system T_5 . The

ultraviolet-absorbing band (R_F , 0.48–0.58) is eluted and the eluate evaporated to afford 102 mg (78%) of compound *V* in the form of a solid foam. On thin-layer chromatography in T_5 , compound *V* affords an elongated spot of the diastereoisomeric mixture (R_F , 0.40–0.52). Successive treatment with methanolic ammonia (2 h at 50°C) and 20% aqueous acetic acid (30 min at 50°C) transforms compound *V* into UpUpU, as confirmed on comparison with an authentic sample.

B. A mixture of the triester *Iib* (0.35 mmol) and the monophosphate *Ia* (0.7 mmol) is coevaporated three times with pyridine, the residue is shaken briefly with 2,3,5-triisopropylbenzenesulfonyl chloride (1.05 g) and pyridine (10 ml), and the resulting mixture is evaporated just to the beginning of crystallisation. The concentrate is kept at room temperature for 6 h, treated with 3-hydroxypropionitrile (0.47 ml), kept for additional 15 h at room temperature, diluted with chloroform (5 ml), and chromatographed on two 20 × 20 × 0.6 cm layers of loose silica gel in the solvent system T_6 . The dimethoxytrityl-group-positive bands (6–17 cm) are eluted, the eluates evaporated, the residue coevaporated three times with toluene, and dissolved at 0°C in 90% aqueous acetic acid (5 ml). After 15 h at 0°C, the solution is evaporated, the residue coevaporated twice with 1-butanol, and chromatographed on three 40 × 16 × 0.6 cm layers of loose silica gel similarly to procedure *A*. Yield, 319 mg (68%) of compound *V*.

2'-O-Acetyluridylyl-(3'→5')-2',3'-di-O-benzoyluridine P-(2-Cyanoethyl) Ester (*VIII*)

A mixture of 2',3'-di-O-benzoyluridine (900 mg) and the monophosphate *Ic* (1 mmol) is coevaporated three times with pyridine, the residue shaken with 2,3,5-triisopropylbenzenesulfonyl chloride (1.5 g) and pyridine (15 ml) for several minutes, and evaporated just to the beginning of crystallisation. The concentrate is kept at room temperature for 5 h, treated with 3-hydroxypropionitrile (0.67 ml), kept for additional 15 h, diluted with chloroform (5 ml), and chromatographed on two 20 × 20 × 0.6 cm layers of loose silica gel in T_4 . The dimethoxytrityl-group-positive bands (12–17 cm) are eluted with T_6 , the eluates evaporated, the residue coevaporated three times with toluene, and dissolved in 90% aqueous acetic acid (20 ml). After 3 h at 20°C, the solution is evaporated, the residue coevaporated twice with 1-butanol, and chromatographed on three layers (40 × 16 × 0.6 cm) of loose silica gel in T_2 . Work-up of the ultraviolet-absorbing bands (R_F , 0.39) afforded 715 mg (84%) of compound *VIII* which is converted to UpU by the action of methanol–conc. aqueous ammonia mixture. An identical product was obtained by reaction of 2',3'-di-O-benzoyluridine (1 mmol), the diester *Id* (0.5 mmol), and 2,3,5-triisopropylbenzenesulfonyl chloride (2 mmol), and the subsequent removal of the dimethoxytrityl group; overall yield, 45%.

2'-O-Acetyluridylyl-(3'→5')-2'-O-acetyluridylyl-(3'→5')-2',3'-di-O-benzoyluridine P¹,P²-bis-(2-Cyanoethyl) Ester (*X*)

A mixture of the triester *VIII* (426 mg; 0.5 mmol) and the phosphate *Ic* (1 mmol) is coevaporated three times with pyridine, the residue shaken with 2,3,5-triisopropylbenzenesulfonyl chloride (1.5 g) and pyridine (10 ml) for several minutes, and evaporated just to the beginning of crystallisation. The concentrate is kept at room temperature for 5 h, treated with 3-hydroxypropionitrile (0.67 ml), kept for additional 20 h, diluted with chloroform (3 ml), and chromatographed on two 20 × 20 × 0.6 cm layers of loose silica gel in the solvent system T_6 . The dimethoxytrityl-group-positive bands (12–17 cm) are eluted with T_6 , the eluates evaporated, the residue coevaporated three times with toluene, and dissolved in 90% aqueous acetic acid (20 ml). After 3 h at room temperature, the solution is evaporated, the residue coevaporated twice with 1-butanol, and chromatographed on two 40 × 16 × 0.6 cm layers of loose silica gel in T_7 . Elution (with T_6) of the

ultraviolet-absorbing bands (R_F , 0.55) afforded 405 mg (65%) of compound *X* (R_F in T_2 , 0.21), which was characterised by the quantitative conversion into UpUpU.

2'-O-Acetyluridylyl-(3'→5')-2'-O-acetyluridylyl-(3'→5')-2'-O-acetyluridylyl-(3'→5')-2',3'-di-O-benzoyluridine P^1, P^2, P^3 -Tris(2-cyanoethyl) Ester (*XII*)

The ester *XII* was prepared analogously to compound *X* from the trinucleotide *X* (330 mg; 0.26 mmol), the phosphate *Ic* (0.5 mmol), 2,3,5-triisopropylbenzenesulfonyl chloride (750 mg), and 3-hydroxypropionitrile (0.3 ml). The final chromatography in T_7 afforded 197 mg (46%) of the ester *XII*, R_F 0.40. Deblocking with the methanol-conc. aqueous ammonia mixture led to UpUpUpU.

Elemental analyses were performed in the Analytical Department (Dr J. Horáček, Head) of this Institute.

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Translated by J. Pliml.