SYNTHESIS OF A URIDINE HEXANUCLEOTIDE DERIVATIVE*

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On repeated treatments with 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate (*I*), 2,3,5-triisopropylbenzenesulfonyl chloride and 2-cyanoethanol and by the subsequent action of 90% aqueous acetic acid, 2′,3′-di-O-benzoyluridine affords the pentanucleotide 2-cyanoethyl ester derivative *V* which is transformed by the action of the phosphate *I* and 2,3,5-triisopropylbenzenesulfonyl chloride followed by heating with ammonia into 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-uridine (*VI*).

In order to develop a more effective and less time-consuming synthesis of ribooligonucleotides, an approach was in this Laboratory examined consisting in the formation of the internucleotide bond by condensation of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranylribonucleoside 3′-phosphates with a component bearing an unprotected $C_{(5')}$-hydroxylic function; the condensation is performed in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride. The thus-obtained phosphodiester is *in situ* converted by the action of 2-cyanoethanol into the phosphotriester which is isolated by preparative thin-layer chromatography on loose silica gel[1]. With the use of pyridine-containing solvent systems, the acidic components of the reaction mixture are completely separated; the product is then purified by precipitation of the chloroform solution with ether. This method has been used in the routine synthesis of numerous analogous of trinucleotides[2,3].

In the present paper we wish to report on the application of the above method to the stepwise synthesis of the derivative *VI* of a uridine-containing hexanucleotide. Further use of this hexanucleotide in the preparation of derivatives bearing groups on the *cis*-diol system required protection of the remaining hydroxylic functions. For this purpose, 2′,3′-di-O-benzoyluridine appeared advantageous as the starting material in view of the content of two alkali-labile protecting groups; 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate[1] (*I*) protected by alkali-stable groupings was used as the prolongation agent. All steps up to the stage of the penta-

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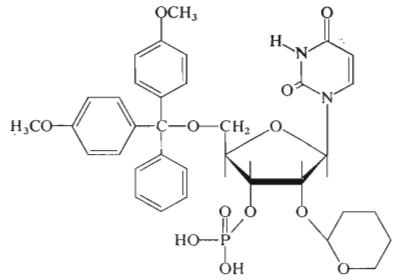
nucleotide *stage* were effected by the combined synthesis; the thus-obtained triester derivative of the pentanucleotide with an unprotected $C_{(5')}$-hydroxyl was then condensed with the phosphate *I*. The reaction product was processed with ammonia to remove the 2-cyanoethyl and benzoyl groups. The product *VI* was isolated by chromatography on DEAE-cellulose and gradient elution with the use of triethylammonium hydrogen carbonate in 50% aqueous ethanol. The product was characterised by deblocking to uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridylyl-(3′ → → 5′)-uridylyl-(3′ → 5′)-uridine exhibiting identical chromatographic and electrophoretic properties as the compound prepared earlier[4] in this Laboratory. The pan-



I [DMTrU(Thp)p]

HO—U(Thp)p(CNEt)UBz$_2$ ⟶ HO—U(Thp)p(CNEt)U(Thp)p(CNEt)UBz$_2$ ⟶

II *III*

HO—U(Thp)p(CNEt)U(Thp)p(CNEt)U(Thp)p(CNEt)UBz$_2$ ⟶

IV

HO—U(Thp)p(CNEt)U(Thp)p(CNEt)U(Thp)p(CNEt)U(Thp)p(CNEt)UBz$_2$ ⟶

V

DMTr—U(Thp)pU(Thp)pU(Thp)pU(Thp)pU(Thp)pU ⟶

VI

HO—U(Thp)pU(Thp)pU(Thp)pU(Thp)pU(Thp)pU ⟶ UpUpUpUpUpU

VII *VIII*

creatic ribonuclease degradation yielded uridine and uridine 3′-phosphate in the expected ratio. With about 40% yields in each step of the combined synthesis and a 14% yield in the final step, the overall yield of product *VI* was 0·26%.

EXPERIMENTAL

Paper chromatography was carried out on papers Whatman in the solvent system S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets or (preparative runs) on the Pitra macroporous silica gel (produced by Service Laboratories of this Institute).

Derivatives *II*—*V*

The hydroxylic component and the protected phosphate *I* (for mmol see Table I) are twice evaporated with about ten parts by weight of pyridine at 20°C/1 Torr. The residue is dissolved in the same volume of pyridine and 2,3,5-triisopropylbenzenesulfonyl chloride (2 equivalents per the phosphate component). The mixture is shaken for several minutes till homogeneous. The solution is concentrated to the consistence of a sirup which is kept at room temperature for 20 h under exclusion of moisture. Pyridine (10 ml per 1 mmol of the phosphate component) and a further portion of 2,3,5-triisopropylbenzenesulfonyl chloride (2 equivalents per the phosphate component) are then added, the whole mixture shaken until homogeneous, concentrated to the consistence of a sirup, and treated with 2-cyanoethanol (16 equivalents per the phosphate component). The mixture is shaken for 15 min, kept at room temperature for 4 h, diluted with an equal volume of chloroform, and the solution chromatographed on layers of loose silica gel (25 × 20 × 0·6 cm; one layer per 1—2 mmol of the phosphate component) in 8 : 2 chloroform–pyridine solvent system. The dimethoxytrityl group-containing region of the layer (identified by pressing a strip of chromatographic paper to the moist layer, brief drying, and spraying with a 10% solution of perchloric acid in 30% aqueous acetic acid) is eluted with 1 : 1 chloroform–methanol solvent system. The eluate is evaporated (40°C, 15 Torr), the residual sirup coevaporated with an equal volume of toluene, the final residue dissolved in chloroform (5—19 ml per 1 mmol of the phosphate component), and the solution precipitated with ten volumes of ether.

TABLE I

Reaction Conditions of the Synthesis of Compounds *II*—*V*, Yields, and R_F Values (in 8 : 2 chloroform–methanol)

5′-Hydroxylic component mmol	Phosphate component mmol	Product	Yield	R_F
3	6	*II*	45	0·79
1·9	4	*III*	37	0·70
0·7	2	*IV*	34	0·61
0·24	1	*V*	33	0·55

The precipitate is separated by decantation and the whole process is repeated once more. The solid is collected with suction (sintered glass filter funnel G 3), washed repeatedly with ether and dried under diminished pressure. The thus-obtained dimethoxytrityl derivative is dissolved in about 20 volumes of 90% aqueous acetic acid and the solution is set aside. The removal of the dimethoxytrityl group is checked by thin-layer chromatography in 9 : 1 chloroform–methanol solvent system. When the reaction is completed (60—90 min), the solution is evaporated (20°C, 1 Torr), the residue coevaporated with a small amount of 1-butanol, and the final residue is dissolved in chloroform (about 10 ml per 0·5 g of the dimethoxytrityl derivative). The solution is added dropwise with stirring into ether (ten volumes with respect to chloroform), the precipitate collected with suction, washed repeatedly with ether, and dried under diminished pressure.

Hexanucleotide Derivative *VI*

A mixture of compound *V* (80 μmol) and the protected phosphate *I* (0·8 mmol) is coevaporated with two portions of pyridine and then dissolved in pyridine (10 ml). 2,3,5-Triisopropylbenzenesulfonyl chloride (1·6 mmol) is added, the mixture shaken for several minutes, and evaporated to the consistence of a sirup which is set aside for 24 h. Water (2 ml) is then added with cooling, the mixture kept at room temperature for 5 min and treated with conc. aqueous ammonia (5 ml) and pyridine (1·5 ml). The solution is kept at 50°C for 1 h and the ammonia is evaporated (40°C, 1 Torr). The residue is applied to a column (500 ml) of DEAE-cellulose (bicarbonate) prewashed with 50% aqueous ethanol. The column is eluted with the use of a linear gradient (4 l. of 50% aqueous ethanol in the mixing chamber and 4 l. of 0·4M triethylammonium hydrogen carbonate in 50% aqueous ethanol in the reservoir). The peak corresponding to 0·3M eluant is evaporated and the residue is coevaporated with three portions of ethanol. The final residue is dissolved in methanol (5 ml), the solution treated with ether (50 ml), and the whole kept at 0°C for 20 h. The precipitate is collected with suction, washed with ether, and dried under diminished presdure to afford 33 mg (14%) of the triethylammonium salt of compound *VI*; R_F value 0·35 (Silufol UV_{254}, S_1).

Deblocking of Compound *VI*

A solution of compound *VI* (3 mg) in 80% aqueous acetic acid (1 ml) is kept at room temperature for 1 h and evaporated (20°C, 1 Torr). The residue is triturated with ether and the precipitate of the tetrahydropyranyl derivative *VII* is washed by decantation with ether (*VII*: R_F 0·1 on Silufol UV_{254} in S_1). Acetic acid (1 ml of a 20% aqueous solution) is added, the mixture heated at 50°C for 2 h, and the resulting solution chromatographed on a 20 cm-wide strip of paper Whatman No 3 MM in the solvent system S_1 for 3 days. The UV-absorbing band R_{Up} 0·17 is eluted with water. The thus-obtained hexanucleotide *VIII* is degraded by pancreatic ribonuclease to uridine 3′-phosphate and uridine in the ratio 5·2 : 1.

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