OLIGONUCLEOTIDIC COMPOUNDS. XLV.*.

CONDENSATION OF THE TRIESTER-CONTAINING BLOCKS IN THE *ribo*-SERIES

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2′-O-Acetyluridine 3′-bis(2,2,2-trichloroethyl) phosphate (*IId*) was converted by a successive treatment with 5′-O-dimethoxytrityl-2′-O-acetyluridine 3′-phosphate (*IIb*) and 3-hydroxypropionitrile in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride and the subsequent reaction with zinc to 5′-O-dimethoxytrityl-2′-O-acetyluridylyl-(3′→5′)-2′-O-acetyluridine 3′-phosphate-[P-(2-cyanoethyl) ester] (*IV*). Compound *IV* was condensed with 2′-O-acetyluridylyl-(3′→5′)-2′,3′-di-O-benzoyluridine[P-(2-cyanoethyl) ester] (*V*) and 3-hydroxypropionitrile to afford the fully protected tetranucleoside triphosphate *VI*.

The synthesis of an uridine-containing hexanucleotide by the N,N′-dicyclohexylcarbodiimide condensation of preformed trinucleotidic blocks containing phosphodiester bonds has been reported in an earlier paper of this Series[1]. The yields of this condensation were very low and did not become satisfactory when 2,3,5-triisopropylbenzenesulfonyl chloride was used as the condensing agent in an analogous synthesis (Ohtsuka and coworkers[2]). The unfavourable influence of internucleotidic bonds on the course of the synthesis is eliminated by the use of blocks, the internucleotidic bonds of which are protected in the form of triesters. The triester synthesis of such dinucleotidic blocks has been reported to afford high yields[3].

The triester synthesis (in spite of higher yields than in the case of the diester synthesis, beginning with the trinucleotidic step) has been observed to afford decreased yields when bulky reaction partners are used. On the basis of this observation, an approach has been proposed consisting in the use of a phosphomonoester in the formation of the new internucleotidic bond and conversion of the thus-obtained diester bond *in situ* into the triester[4]. This approach which makes use of the higher reactivity of phosphomonoesters in comparison with that of phosphodiesters, could lower the unfavourable effects of steric factors in reactions of bulky molecules.

This principle (named "combined synthesis") is used in the present paper to connect the triester-bond-containing dinucleotidic blocks. The main problem of this approach

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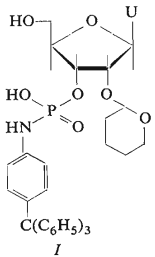
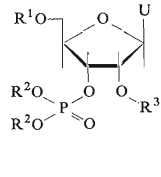
consists in the synthesis of the dinucleoside (2-cyanoethyl) phosphate bearing a primary phosphoryl group. With the use of protected 5′-O-dimethoxytritylribonucleoside 3′-phosphates as active components, this problem is limited to the preparation of a compound bearing a free $C_{(5')}$-hydroxylic function and a protected phosphoryl group at position $C_{(3')}$. This phosphoryl group must be protected in such a manner to allow its deblocking without endangering the alkalilabile 2-cyanoethyl-group-containing triester and the acidolabile dimethoxytrityl group. Protection of the phosphoryl group in the form of a *p*-triphenylmethylanilidate[5] represented one of the possible routes. Unfortunately, 2′-O-tetrahydropyranyluridine 3′-(*p*-triphenylmethyl) anilidate (*I*), prepared analogously to the reported procedure[5], did not afford a satisfactory yield of the protected dinucleotide in the triester condensation with the protected uridine 3′-(2-cyanoethyl) phosphate, probably because of the side reactions on the phosphoamidate group. Another route was therefore used consisting in the complete protection of the phosphoryl group in the form of a triester with 2,2,2-trichloroethanol. The starting component of this type, namely, 2′-O-tetrahydropyranyluridine 3′-bis(2,2,2-trichloroethyl) phosphate (*IIa*) was prepared by two procedures. In one of them, the $C_{(3')}$-hydroxylic function of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine was phosphorylated with bis(2,2,2-trichloroethyl) phosphochloridate. In spite of the selectivity of this phosphorylating agent for the $C_{(5')}$-hydroxylic function[6], the reaction afforded about 50% of the 3′-phosphoryl derivative; removal of the dimethoxytrityl group from the latter derivative furnished compound *IIa*. As shown by preliminary tests on the removal of the 2,2,2-trichloroethyl groups with zinc in a mixture of pyridine and acetic acid, the reaction proceeds unequivocally and the tetrahydropyranyl group remains intact.



I

U = uracil



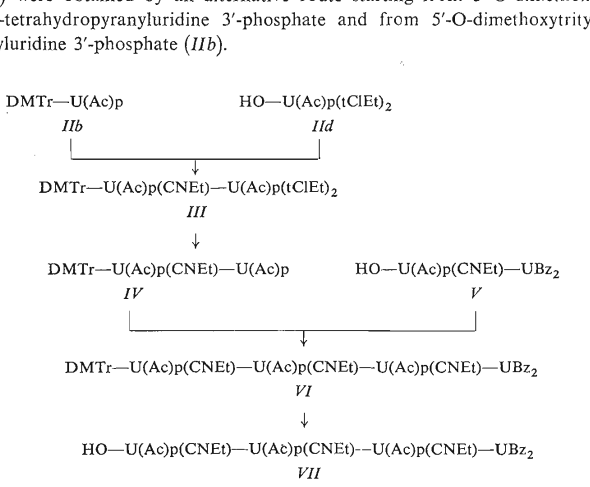
IIa; R^1, = H, $R^2 = CH_2CCl_3$, R^3 = 

IIb; $R^1 = C(C_6H_5)(C_6H_3OCH_3)_2$, R^2 = H, $R^3 = COCH_3$

IIc; $R^1 = C(C_6H_5)(C_6H_4OCH_3)_2$, $R^2 = CH_2CCl_3$, $R^3 = COCH_3$

IId; $R^1 = R^1 = H$, $R^2 = CH_2CCl_3$, $R^3 = COCH_3$

Another route how to prepare compound *IIa* and the 2′-O-acetyl analogue *IId* consisted in the double esterification of the corresponding 3′-phosphates with 2,2,2-trichloroethanol in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride. In this manner, the pyridinium salt of 5′-O-acetyl-2′-O-tetrahydropyranyluridine 3′-phosphate afforded an 85% yield of the bis(2,2,2-trichloroethyl) ester. In the course of the removal of the 5′-O-acetylgroup with a mixture of methanol and conc. aqueous ammonia, the phosphotriester bearing the two 2,2,2-trichloroethyl groups proved relatively labile in alkaline media. Even after one hour, the reaction affords the phosphodiester as the predominant product and the yield of the triester *IIa* is only 27%. Somewhat higher yields (36% of compound *IIa* and 40% of compound *IId*, resp..) were obtained by an alternative route starting from 5′-O-dimethoxytrityl--2′-O-tetrahydropyranyluridine 3′-phosphate and from 5′-O-dimethoxytrityl-2′-O--acetyluridine 3′-phosphate (*IIb*).



SCHEME 1

The block synthesis from the acetyl derivative *IId* as the starting compound is shown on Scheme 1. In the first step, the phosphate *IIb* was condensed with the triester *IId* and the thus-obtained diester intermediate was converted *in situ* to the triester *III* in 66% yield. Removal of the 2,2,2-trichloroethyl groups with zinc in a mixture of pyridine and acetic acid[7] afforded the triester *IV* bearing a free phosphoryl group. Condensation of compound *IV* with 2′-O-acetyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyl-uridine [P-(2-cyanoethyl) ester] (*V*) and 3-hydroxypropionitrile led to the fully protected tetranucleoside triphosphate *VI* in 77% yield.

EXPERIMENTAL

The preparative and analytical thin-layer chromatography was performed analogously to the earlier paper[10] in the solvent systems T_1, chloroform–methanol–pyridine (90 : 5 : 5); T_2, chloroform–methanol (95 : 5); T_3, chloroform–methanol (9 : 1); T_4, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2); T_5, chloroform–methanol–pyridine (70 : 15 : 15); T_6 chloroform–methanol (8 : 2); and T_e, chloroform–methanol (1 : 1).

2′-O-Tetrahydropyranyluridine 3′-Phosphoro-(*p*-triphenylmethyl) Anilidate (*I*)

A. The anhydrous solution of the pyridinium salt of 5′-O-acetyl-2′-O-tetrahydropyranyluridine 3′-phosphate[8] (2 mmol) in pyridine (20 ml) is treated with N,N′-dicyclohexylcarbodiimide (4 g) and kept at room temperature for 10 min. A solution of triphenyl-*p*-aminophenylmethane (1·35 g) in pyridine (10 ml) is then added and the whole mixture is concentrated under diminished pressure to the volume of about 10 ml, the mixture is kept at room temperature for 3 days, diluted with an equal volume of water, kept for additional 15 h, filtered, and the material on the filter washed with 50% aqueous pyridine. The filtrate and washings are combined, evaporated under diminished pressure, and the residue coevaporated with two portions of pyridine. The residue is dissolved in chloroform (100 ml), the solution is washed with two 25 ml portions of 0·3M triethylammonium hydrogen carbonate, and evaporated. The residue is kept under a mixture of toluene (50 ml) and n-heptane (50 ml) for 15 h to deposit a solid which is collected with suction, washed on the filter with the same solvent mixture, and air-dried. The thus-obtained 5′-O-acetyl derivative of compound *I* is dissolved in pyridine (10 ml), the solution treated with 15% methanolic ammonia, kept at room temperature for 15 h, and evaporated under diminished pressure. The residue is chromatographed on two 16 × 40 × 0·6 cm layers of loose silica gel in T_6. The UV-absorbing bands (R_F value about 0·25) are eluted with T_e and the eluate is evaporated to afford 760 mg (45%) of the triethylammonium salt of compound *I* in the form of a solid foam. For $C_{39}H_{41}N_3O_{10}P.C_6H_{15}N$ (834·5) calculated: 6·72% N; found: 7·23% N. R_F value: 0·32 (in T_3) and 0·72 (in T_4). The product is converted by the action of isopentyl nitrite into 2′-O-tetrahydropyranyluridine 3′-phosphate.

B. To an anhydrous solution of the pyridinium salt of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate (1 mmol) in pyridine (10 ml), there is added N,N′-dicyclohexylcarbodiimide (1 g) and the mixture is kept at room temperature for 1 h. A solution of triphenyl-*p*-aminophenylmethane (700 mg) in pyridine (10 ml) is then added, the whole mixture is concentrated to the volume of about 5 ml under diminished pressure, the concentrate is kept at room temperature for 3 days, filtered, and the filtrate chromatographed on three 20 × 20 × 0·6 cm layers of loose silica gel in T_3. The dimethoxytrityl-positive bands (distance from the start line, 4—9 cm) are eluted with T_e, the eluate is evaporated, and the residue is coevaporated with two portions to toluene. The final residue is dissolved in 90% aqueous acetic acid, the solution kept at 0°C for 15 h, and evaporated under diminished pressure. The residue is coevaporated with 1-butanol and chromatographed on two 16 × 40 × 0·6 cm layers of loose silica gel in T_3. The UV-absorbing band (R_F 0·40) is eluted with T_e containing 0·16 ml of triethylamine and the eluate is evaporated to afford 302 mg (36%) of compound *I*.

2′-O-Tetrahydropyranyluridine 3′-Bis(2,2,2-trichloroethyl) Phosphate (*IIa*)

A. An anhydrous solution of the pyridinium salt of 5′-O-acetyl-2′-O-tetrahydropyranyluridine 3′-phosphate (1 mmol) in pyridine (5 ml) is shaken with 2,3,5-triisopropylbenzenesulfonyl chloride (1·2 g) for 30 min. 2,2,2-Trichloroethanol (0·4 ml) is then added, the whole mixture kept at room temperature for 20 h, diluted with 1M sodium acetate (20 ml), and extracted with two 25 ml portions of chloroform. The extract is dried over anhydrous magnesium sulfate,

evaporated, the residue coevaporated with two portions of toluene, and chromatographed on two 40 × 16 × 0·6 cm layers of loose silica gel in T_3. The UV-absorbing bands (R_F 0·15 to 0·40) are eluted with T_e and the eluate is evaporated to afford 606 mg of the crude 5′-O-acetyl derivative of compound *IIa* which is dissolved in methanol (5 ml), the solution diluted with conc. aqueous ammonia (5 ml), kept at room temperature for 1 h, and evaporated. The residue is chromatographed on two 40 × 16 × 0·6 cm layers of loose silica gel in T_3. Elution of the UV-absorbing bands (R_F 0·30) with T_e and evaporation of eluates affords 180 mg (27%) of compound *IIa*. For $C_{15}H_{23}Cl_6N_2O_{10}P$ (671·1) calculated: 28·64% Cl, 3·63% N; found: 27·92% Cl, 3·51% N. R_F value in T_3: 0·32.

B. To an anhydrous solution of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine[9] (1·4 g; 2·2 mmol) in pyridine (5 ml) there is added bis(2,2,2-trichloroethyl) phosphochloridate (0·9 g), the reaction mixture is kept at room temperature for 20 h, diluted with chloroform (3 ml), and chromatographed on two 20 × 20 × 0·6 cm layers of loose silica gel in T_2. The dimethoxytrityl-positive bands (R_F value of about 0·75) are eluted with T_e and evaporated to afford 1·06 g of the 5′-O-dimethoxytrityl derivative of compound *IIa*. This derivative is dissolved at 0°C in 90% aqueous acetic acid (20 ml), the solution kept at 0°C for 20 h, evaporated under diminished pressure, the residue coevaporated with two portions of 1-butanol, and chromatographed on two 40 × 16 × 0·6 cm layers of loose silica gel in T_3. Elution of the UV-absorbing bands (R_F 0·40) and evaporation of eluates affords 430 mg (36%) of compound *IIa*.

2′-O-Acetyluridine 3′-bis(2,2,2-Trichloroethyl) Phosphate (*IId*)

To an anhydrous solution of the pyridinium salt of 5′-O-dimethoxytrityl-2′-O-acetyluridine 3′-phosphate (*IIb*; 4 mmol) there is added 2,3,5-triisopropylbenzenesulfonyl chloride (6 g), the reaction mixture is shaken for 30 min, and concentrated to the beginning of crystalisation. 2,2,2-Trichloroethanol (4 ml) is then added to the concentrate, the whole mixture kept at room temperature for 30 h, diluted with chloroform (10 ml), and chromatographed on 3 layers (40 × × 16 × 0·6 cm) of loose silica gel in T_2. The UV-absorbing bands (R_F 0·30) are eluted with T_e and the eluates are evaporated to afford 1·056 g (40%) of compound *IId*. R_F value: 0·51 (in T_2). For $C_{15}H_{17}Cl_6N_2O_{10}P$ (629·0) calculated: 33·62% Cl, 4·45% N; found: 32·90% Cl, 4·32% N.

5′-O-Dimethoxytrityl-2′-O-acetyluridylyl-(3′ → 5′)-2′-O-acetyluridine 3′-Bis(2,2,2-trichloroethyl) Phosphate [P-(2-Cyanoethyl) Ester] (*III*)

To an anhydrous solution of the phosphate *IIb* (2 mmol) and the triester *IId* (1·5 mmol) in pyridine (10 ml) there is added 2,3,5-triisopropylbenzenesulfonyl chloride (3·0 g), the reaction mixture is shaken for several minutes and then concentrated under diminished pressure just to the beginning of crystallisation. The concentrate us kept at room temperature for 5 h, treated with 3-hydroxypropionitrile (1·35 ml). kept for additional 20 h, diluted with chloroform (5 ml), and chromatographed on three 20 × 20 × 0·6 cm layers of loose silica gel in T_1. The dimethoxytrityl-positive bands (10·5—17·0 cm) are eluted with T_e, the eluates evaporated, the residue coevaporated with two portions of toluene, and chromatographed on two 40 × 16 × 0·6 cm layers of loose silica gel in T_2. Elution of the UV-absorbing and dimethoxytrityl-positive bands (R_F 0·33) with T_e and evaporation of eluates affords a solid foam of compound *III* (1·22 g; 66%), R_F 0·38 (in T_3). For $C_{50}H_{50}Cl_6N_5O_{18}P$ (1 252·6) calculated: 16·88% Cl, 5·59% N; found: 16·35% Cl, 5·32% N.

5′-O-Dimethoxytrityl-2′-O-acetyluridylyl-(3′→ 5′)-2′-O-acetyluridine 3′-Phosphate [P^1-(2-Cyanoethyl) Ester] (*IV*)

A solution of compound *III* (1·25 g; 1 mmol) in a mixture of pyridine (6 ml) and acetic acid (2 ml) is stirred with powdered zinc (5 g) for 2 h. A thick suspension of pyridinium Dowex 50 ion exchange resin (5 ml) in water is then added, the whole kept at room temperature for 5 min, and applied to the top of a column (10 ml) packed with the same resin. The column is eluted with 50% aqueous pyridine (50 ml) and the effluent is taken at 0°C. The effluent is then evaporated at 15°C/1 Torr and the residue is coevaporated with four portions of pyridine. The final residue is dissolved in pyridine (10 ml) and the solution is added dropwise into ether (300 ml) with stirring. The precipitate is collected with suction, washed with ether, and dried under diminished pressure to afford 900 mg (72%) of the pyridinium salt of compound *III*, R_F 0·05 (in T_3). The product is characterised by conversion into UpUp (ref.[10]) by successive treatments with 80% aqueous acetic acid and methanolic ammonia (R_{Up} 0·58 in T_4).

5′-O-Dimethoxytrityl-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] (*VI*)

To an anhydrous solution of compound *IV* (0·08 mmol) and 2′-O-acetyluridylyl-(3′→ 5′)-di-O-benzoyluridine [P-(2-cyanoethyl) ester] (ref.[11]) (*V*; 0·16 mmol) in pyridine (3 ml) there is added 2,3,5-triisopropylbenzenesulfonyl chloride (120 mg). The whole mixture is shaken for several minutes and evaporated under diminished pressure to the consistence of a thick sirup. The sirup is kept at room temperature for 20 h, treated with 3-hydroxypropionitrile (0·05 ml), kept for additional 20 h, diluted with chloroform (2 ml), and chromatographed on a 20 × 20 × 0·6 cm layer of loose silica gel in T_5. The dimethoxytrityl-positive band (10—17 cm) is eluted with T_e, the eluate evaporated, the residue coevaporated with two portions of toluene, and rechromatographed on a 40 × 16 × 0·6 cm layer of loose silica gel in T_3. Elution of the UV-absorbing and dimethoxytrityl-positive band (R_f 0·35) and evaporation of the eluate affords 121 mg (77%) of compound *VI* which is characterised by conversion into compound *VII* on treatment with 90% aqueous acetic acid (compound *VII* was prepared by the stepwise synthesis[10]).

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REFERENCES

1. Smrt J., Šorm F.: This Journal *29*, 2971 (1964).
2. Ohtsuka E., Ubasawa M., Ikehara M.: J. Am. Chem. Soc. *93*, 2296 (1971).
3. Werstiuk E. S., Neilson T.: Can. J. Chem. *50*, 1283 (1972).
4. Smrt J.: This Journal, in press.
5. Agarwal K. I., Yamazaki A., Khorana H. G.: J. Am. Chem. Soc. *93*, 2754 (1771).
6. Franke A., Scheit K. - H., Eckstein F.: Chem. Ber. *101*, 2998 (1968).
7. Eckstein F., Rizk I.: Chem. Ber. *102*, 2362 (1969).
8. Smrt J. in the book: *Synthetic Procedures in Nucleic Acid Chemistry* (W. W. Zorbach, T. D. Tipson, Eds), p. 466. Interscience, New York 1968.
9. Smrt J., Malkievicz A.: This Journal *38*, 2962 (1973).
10. Smrt J., This Journal *38*, 3189 (1973).

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