COMBINED SYNTHESIS OF OLIGONUCLEOTIDES IN THE DEOXY SERIES*

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5'-O-Dimethoxytritylthymidine (*I*) reacts with 3'-O-acetylthymidine 5'-phosphate (*II*) in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride under the formation of a dinucleoside phosphate which is converted *in situ* by the action of 2-(phenylthio)ethanol to the completely protected triester *IIIa*; on treatment with methanolic ammonia, compound *IIIa* affords 5'-O-dimethoxytritylthymidylyl-(3' → 5')-thymidine [P-(2-phenylthioethyl) ester] (*IIIb*). Compound *IIIb* and *II* afford similarly 5'-O-dimethoxytritylthymidylyl-(3' → 5')-thymidylyl-(3' → 5')-3'-O-acetylthymidine [P^1,P^2-bis(2-phenylthioethyl) ester] (*IV*). On the successive treatment with periodic acid in pyridine and then 2M-NaOH, compounds *IIIb* and *IV* afford TpT and TpTpT, respectively.

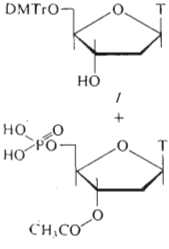
In this Laboratory, a new approach[1] to the synthesis of the oligonucleotidic chain has been developed, promising a considerable improvement of yields in the particular steps and shortening of the otherwise time-consuming isolation processes. The most important points of the procedure are as follows: *a*) the active component is represented by a protected ribonucleoside 3'-phosphate bearing at position $C_{(5')}$ a dimethoxytrityl groups as a transient protecting group and simultaneously, as a marker of the product during the isolation process; *b*) the intermediary phosphodiester is *in situ* converted to the triester by reaction with 2-cyanoethanol; *c*) the neutral triester is isolated on a thick layer of silica gel in a pyridine-containing solvent system and identified with the use of the dimethoxytrityl marker; and *d*) after the removal of the transient protecting group, the intermediate with a protected internucleotidic bond enters the further diester condensation. In the *ribo* series, the active component is represented by a $C_{(3')}$-phosphate. It was of interest to attempt the combined synthesis with the use of a $C_{(5')}$-phosphate in the role of the active component. The most accessible starting material for this type of synthesis may be found in the deoxy series.

In the present paper, we wish to report the synthesis of a TpTpT derivative, starting from 5'-O-dimethoxytritylthymidine (*I*) and 3'-O-acetylthymidine 5'-phosphate (*II*).

* Part IL in the series Oligonucleotidic Compounds; Part XLVIII: This Journal *39*, 969 (1974).

In the isolation of intermediates, the dimethoxytrityl group was again used as marker, representing of course the permanent protecting group. Acetyl is used as the transient protecting group. Conditions required in removal of the acetyl group exclude the use of the 2-cyanoethyl group for the protection of the internucleotidic bond. The 2-phenylthio group appeared to possess somewhat more promising properties in this respect; this group has been recently used by Narang and coworkers to protect the primary phosphoryl group. It was questionable, however, if the alkali stability of this group in a triether would be similar to that in a phosphodiester[2].

In the first step, 1 equivalent of 3'-O-acetylthymidine 5'-phosphate pyridinium salt (*II*) was treated with 2 equivalents of 5'-O-dimethoxytritylthymidine (*I*) in the presence of 5 equivalents of 2,3,5-triisopropylbenzenesulfonyl chloride. After four hours, 2-(phenylthio)ethanol (5 equivalents) was added. After additional 20 h, the reaction mixture was subjected to thick layer chromatography on silica gel in the solvent system 90 : 5 : 5 chloroform–methanol–pyridine. A dimethoxytrityl-positive band was obtained containing the product *IIIa* along with the starting hydroxylic component *I* which had been used in excess. As shown by analytical thin-layer chromatography, the R_F values of compounds *I* and *IIIa* are too similar to allow an efficient chromatographic separation. The mixture was therefore processed with methanolic ammonia to convert compound *IIIa* to *IIIb* (compound *I* remained unchanged). The resulting mixture was then readily separated on a thin layer of silica gel to afford 5'-O-dimethoxytritylthymidylyl-(3' → 5')-thymidine [P-(2-phenylthioethyl) ester] (*IIIb*) in 95% yield. Under conditions of an ammonolysis, the phospho-



I

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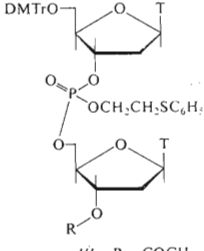
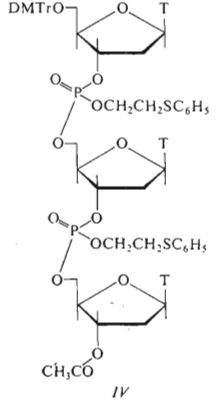
II



IIIa, R = $COCH_3$
IIIb, R = H



IV

triester bond did not suffer any cleavage. Consequently, the 2-phenylthioethyl group is sufficiently stable also in a phosphotriester.

Compound *IIIb* was subjected to a further reaction with compound *II* and 2-(phenylthio)ethanol. In this step (in contrast to the first step), an excess is used of the phosphate component *II* which is converted to a bis(2-phenylthioethyl) ester under conditions of the combined synthesis. Thin-layer chromatography of the reaction mixture afforded a dimethoxytrityl-positive band containing exclusively the product *IV* which was isolated in 81% yield. The use of longer reaction periods of time did not lead to improved yields. Notwithstanding, the yields of the present time-saving method are satisfactory.

As reported earlier[2] in the case of phosphodiesters, the 2-(phenylthio)ethyl group was removed by oxidation of the disulfidic sulfur with aqueous sodium metaperiodate and the subsequent β-elimination of the thus-modified protecting group with 2M--NaOH. In the present case of water-insoluble phosphotriesters, the original procedure was modified by the use of periodic acid in pyridine as the oxidating agent. The removal of the 2-(phenylthio)ethyl group was followed by detritylation affording as the single product TpT (from *IIIb*) and TpTpT (from *IV*).

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent system T_1, chloroform–methanol–pyridine (90 : 5 : 5); T_2, chloroform–methanol (9 : 1); and T_3, chloroform–methanol (95 : 5). The preparative chromatography was carried out on a 6 mm thick layer of loose fluorescent-indicator--containing silica gel according to Pitra (produced by Service Laboratories of this Institute in Prague - Suchdol). The dimethoxytrityl derivatives on the preparative layers were detected by a spray with 10% perchloric acid in 30% aqueous acetic acid on a strip of paper Whatman No 1, pressed to the moist layer. Elution of compounds was performed with the solvent mixture T_e chloroform–methanol (1 : 1). Electrophoresis was performed on paper Whatman No 1 immersed in tetrachloromethane with the use of 0·05M triethylammonium hydrogen carbonate buffer solution (pH 7·5). Condensation mixtures were taken down on a rotatory evaporator at 20°C/1 Torr and the atmospheric pressure was restored through a column of silica gel.

5′-O-Dimethoxytritylthymidylyl-(3′→ 5′)-thymidine [P-(2-Phenylthioethyl)Ester] (*IIIb*)

A mixture of 3′-O-acetylthymidine 5′-phosphate pyridinium salt[3] (*II*; 242 mg; 0·5 mmol) and 5′-dimethoxytritylthymidine[4] (*I*; 550 mg; 1 mmol) is coevaporated with three 5 ml portions of pyridine and the final residue is dissolved in pyridine (10 ml). 2,3,5-Triisopropylbenzenesulfonyl chloride (750 mg; 2·5 mmol) is then added, the whole mixture shaken for several minutes, evaporated to the incipient crystallisation, and kept at room temperature for 4 h. 2-(Phenylthio)-ethanol (0·45 ml; 2·5 mmol) is the added, the whole kept for additional 20 h, diluted with chloroform (5 ml) and chromatographed on two 40 × 20 × 0·6 cm layers of loose silica gel in the solvent system T_1. The broad dimethoxytrityl-positive band (distance, 15—24 cm) is eluted with the solvent mixture T_e, evaporated at 30°C/15 Torr, and the pyridine-containing residue is dissolved in 4M methanolic ammonia (20 ml). After 15 h at room temperature, the mixture is

evaporated, the residue coevaporated with toluene (10 ml), and chromatographed on two 40 × × 20 × 0·6 cm layers of silica gel in T_2. Two dimethoxytrityl-positive bands are obtained. The faster one (R_F 0·62) affords by elution (T_e) and evaporation 256 mg of the starting material *I*. The slower band (R_F 0·45) affords 467 mg (95%) of compound *III*, R_F 0·34 (in T_3). For $C_{49}H_{53}N_4O_{14}PS$ (985·0) calculated: 5·69% N, 3·14% P, 3·25% S; found: 5·58% N, 3·57% P, 3·45% S.

5′-O-Dimethoxytritylthymidylyl-(3′→5′)-thymidylyl-(3′→5′)-3′-O-acetylthymidine [P^1,P^2-bis(2-Phenylthioethyl) Ester] *IV*)

The pyridinium salt *II* (193 mg; 0·4 mmol) and compound *IIIb* (207 mg; 0·2 mmol) are coevaporated three times with pyridine, the final residue is dissolved in pyridine (5 ml), and then 2,3,5-triisopropylbenzenesulfonyl chloride (480 mg; 1·6 mmol) is added. The whole mixture is shaken for several minutes, evaporated to the incipient crystallisation, and kept at room temperature for 4 h. 2-(Phenylthio)ethanol (0·4 ml) is then added, the mixture kept for additional 20 h, diluted with chloroform (3 ml), and chromatographed on a layer (40 × 20 × 0·6 cm) of loose silica gel in the solvent system system T_1. The dimethoxytrityl-positive band (R_F 0·50) is eluted with the solvent mixture T_e, the eluate evaporated, the residue coevaporated with toluene, and dried under diminished pressure to afford 238 mg (81%) of compound *IV*, R_F 0·20 (in T_3). For $C_{69}H_{76}N_6O_{22}P_2S_2$ (1 467) calculated: 5·73% N, 4·22% P, 4·37% S; found: 5·53% N, 4·15% P, 4·48% S.

Deblocking of Compounds *IIIb* and *IV*

The protected oligonucleotide (0·01 mmol) is added to a solution of periodic acid (0·05 mmol) in pyridine (0·1 ml), the whole is kept at room temperature for 3 h, and evaporated under diminished pressure. The residue is shaken with 2M-NaOH (0·2 ml) for 2 h. The mixture is adjusted to pH 2 by the addition of Dowex 50 (H^+) ion exchange resin and ethanol (0·1 ml), set aside for 1 h, filtered, and the filtrate evaporated. As shown by electrophoresis in E_1, compound *IIIb* affords TpT (E_{Up} 0·40) while TpTpT (E_{Up} 0·64) is obtained from compound *IV*.

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

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