POLYCONDENSATION OF A THYMIDINE DINUCLEOTIDE CONTAINING PROTECTED INTERNUCLEOTIDIC BOND*

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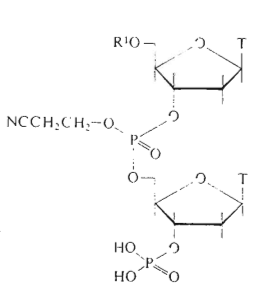
Received March 14th, 1974

Thymidylyl-(3′→ 5′)-thymidine 3′-phosphate [P[1]-(2-cyanoethyl ester] (*I*) in admixture with 18% of 5′-O-dimethoxytritylthymidylyl-(3′→ 5′)-thymidine 3′-phosphate [P[1]-(2-cyanoethyl) ester] (*II*) was condensed by the action of either N,N′-dicyclohexylcarbodiimide or 2,3,5-triisopropylbenzenesulfonyl chloride.

In an earlier paper of this series, there has been reported[1] the synthesis of a thymidine dinucleotide derivative, the internucleotidic bond of which is protected in the form of a triester, namely, the synthesis of 5′-O-dimethoxytritylthymidylyl-(3′ → 5′)--thymidine 3′-phosphate [P[1]-(2-cyanoethyl) ester] (*II*). By removal of the dimethoxytrityl group, compound *II* is converted to thymidylyl-(3′ → 5′)-thymidine 3′-phosphate [P[1]-(2-cyanoethyl) ester] (*I*) which represents the first difunctional dinucleotide derivative with a protected internucleotidic bond. It appears of interest to examine the effect of the protection of the internucleotidic bond on the course of the poly-



I, R^1 = Dimethoxytrityl
II, R^1 = H

* Part LI in the series Oligonucleotidic Compounds; Part L: This Journal *39*, 2483 (1974).

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condensation and furthermore, to compare the products obtained with the use of N,N′-dicyclohexylcarbodiimide on the one hand and 2,3,5-triisopropylbenzensulfonyl chloride on the other (this sulfonyl chloride has not been so far used in polycondensations). The polymerisation of dinucleotides with an unprotected internucleotidic bond has been paid considerable attention in the preparation of polynucleotides with a repeating dinucleotide sequence[2]. As the condensing agent, N,N′-dicyclohexylcarbodiimide has been exclusively used.

In the present work, polycondensations were performed of a mixture of the difunctional component *I* with 18% of the monofunctional component *II*. In condensations with N,N′-dicyclohexylcarbodiimide (10 equivalents), the pyrophosphates are split with acetic anhydride and the removal of 2-cyanoethyl groups and the dimethoxytrityl group is followed by separation of products by means of preparative paper chromatography. The identification was effected on the basis of chromatographical mobility[3] and alkaline phosphatase degradation (Table I). The overall yield was 53% of the nucleotidic material introduced into the reaction.

With 2,3,5-triisopropylbenzenesulfonyl chloride (10 equivalents) as the polycondensation agent, the reaction mixture was processed with 2-cyanoethanol in order

TABLE I
Products of Polycondensation

R_{Up}[a]	O. D.$_{260}$	Rel. %	Substance
		with N,N′-dicyclohexylcarbodiimide	
0·0	19	0·8	d-$(Tp)_8$ and higher
0·30	79	3·4	d-$(Tp)_6$
0·50	95	4·1	cycl.-d-$(Tp)_6$
0·66	150	6·5	cycl.-d-$(Tp)_6$ d-$(Tp)_4$ [21 : 79]
0·80	235	10·0	d-$(Tp)_4$
1·0	286	12·5	cycl.-d-$(Tp)_4$ and d-$(Tp)_2$ [72 : 28]
1·35	1 440	62·5	cycl.-d-$(Tp)_2$
		with 2,3,5-triisopropylbenzenesulfonyl chloride	
0·0	55	2·7	d-$(Tp)_8$ and higher
0·30	168	7·7	d-$(Tp)_6$
0·50	115	5·6	cycl.-d-$(Tp)_6$
0·66	180	8·8	d-$(Tp)_4$
0·80—1·0	410	20	cycl.-d-$(Tp)_4$ and d-$(Tp)_2$ [55 : 45]
1·30	1 120	55	cycl.-d-$(Tp)_2$

[a] Whatman 3 MM.

to cleave the pyrophosphates[4] and to convert the free phosphodiester and phosphomonoester functions into triesters. The acidic removal of the dimethoxytrityl group was followed by cleavage of 2-cyanoethyl esters with aqueous–ethanolic ammonia at 50°C. The final products were separated by preparative paper chromatography as above. The overall yield was 48% of the nucleotidic material introduced into the reaction. For details see Table I.

As it may be seen from the above results, the use of a dinucleotide with a protected internucleotidic bond in polycondensations does not result in increased yields of polynucleotides[2]. About 50% of the resulting nucleotidic material is in the form of a cyclic dinucleotide. The amount of polynucleotides from the hexanucleotide to higher nucleotides is about 4·4% with the use of N,N'-dicyclohexylcarbodiimide and about 10% with the use of 2,3,5-triisopropylbenzenesulfonyl chloride.

EXPERIMENTAL

Descending paper chromatography was performed on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), and S_2, 1-propanol–conc. aqueous ammonia–water (55 : 10 : 35). Because of the somewhat coloured chromatogram, the oligonucleotides were detected on a separated portion of the chromatogram by a tungstate spray (instead of viewing under ultraviolet light). The bacterial alkaline phosphatase (Type III) was the product of Sigma Chemical Company, St. Louis, Missouri, USA.

Thymidylyl-(3′ → 5′)-thymidine 3′-Phosphate [P^1-(2-Cyanoethyl) Ester] (*I*) Pyridinium Salt

A solution of 5′-O-dimethoxytritylthymidylyl-(3′ → 5′)-thymidine 3′-phosphate [P^1-(2-cyanoethyl) ester] (*I*) pyridinium salt (0·7 g) in 90% aqueous acetic acid (30 ml) is kept at 20°C for 90 min and evaporated at 15°C/1 Torr. The residue is coevaporated with three portions of 1-butanol and finally dissolved in pyridine (10 ml). The solution is added dropwise with stirring into ether (600 ml), the precipitate collected by centrifugation, washed with three portions of ether, and dried over phosphorus pentoxide under diminished pressure to afford 0·43 g of the pyridinium salt of compound *I*. Molecular weight 890 as determined from extinction at 260 nm of the TpTp spot (R_{Up} 0·60) eluate after work-up with ammonia (20°C/1 h) and chromatography in S_1; theoretical value for the monopyridinium salt: 758

Polycondensation by means of N,N'-Dicyclohexylcarbodiimide

A mixture of compound *I* (40 mg; 0·04 mmol) and compound *II* (200 mg; 0·22 mmol) is coevaporated three times with pyridine. N,N'-Dicyclohexylcarbodiimide (0·5 g) and pyridine (10 ml) are then added and the whole mixture is evaporated until non-homogeneous. The residue is made homogeneous by the dropwise addition of pyridine, kept at room temperature for 90 h, diluted with 30% aqueous pyridine (30 ml), and washed with cyclohexane (20 ml). The aqueous phase is evaporated under diminished pressure, the residue coevaporated with two portions of pyridine, and finally dissolved in pyridine (10 ml). The solution is treated with acetic anhydride (5 ml), kept at room temperature for 20 h, diluted with water (3 ml), kept for additional 2 h, and evaporated. The residue is dissolved in a mixture of concentrated aqueous ammonia (10 ml) and methanol (10 ml), the whole kept at room temperature for 30 min, diluted with ethanol (20 ml), and evaporated to the consistence of a sirup which is dissolved in 90% aqueous acetic acid. After 1 h, the solution is evaporated at 20°C/1 Torr, the residue coevaporated with two portions of 1-butanol,

and finally dissolved in 50% aqueous ethanol. The solution was chromatographed on one sheet of paper Whatman No 3 MM in the solvent system S_2. A strip of the chromatogram was cut off and the phosphate spots were detected by a tungstate spray. The corresponding bands on the original chromatogram were eluted with 1% aqueous ammonia. The eluates were diluted with ethanol, concentrated, and the concentrates stored at 0°C. The samples (20 $O.D._{260}$ in 0·1 to 0·2 ml each) of particular fractions were incubated with alkaline phosphatase (0·01 ml) in Tris-HCl buffer solution (0·25 ml, pH 9) at 37°C for 2 h and then chromatographed along with original fractions. The products were identified on the basis of chromatographical mobility of the original fractions and dephosphorylation products with the use of synthetically prepared TpTpTpTp and TpTpTpT (*cf.*[1]) as reference specimens (for the results see Table I).

Polycondensation by means of 2,3,5-Triisopropylbenzenesulfonyl Chloride

The same amounts of compounds *I* and *II* as in the preceding paragraph were coevaporated with three portions of pyridine, the residue treated with 2,3,5-triisopropylbenzenesulfonyl chloride (0·7 g) and pyridine (10 ml), the whole shaken for several minutes, and evaporated to incipient crystallisation. The concentrate was kept at room temperature for 72 h, treated with 2-cyanoethanol (0·34 ml), kept for additional 20 h, cooled down to −40°C, and diluted with pyridine (8 ml) and triethylamine (1·1 ml). After 5 min, the mixture was diluted with water (8 ml), kept at room temperature for 20 h, and evaporated at 20°C/1 Torr. The residue was coevaporated with toluene and dissolved in 90% aqueous acetic acid. After 1 h at 20°C, the solution was evaporated at 20°C/1 Torr, the residue dissolved in pyridine (10 ml), the solution evaporated, and the final residue dissolved in a mixture of conc. aqueous ammonia–ethanol (1 : 1; 5 ml). The solution was heated at 50°C for 30 min and then chromatographed on two sheets of paper Whatman No 3 MM in the solvent system S_2. The phosphorus-containing bands were identified (tungstate spray) on a cut-off strip of the chromatogram, eluted with 1% aqueous ammonia, the eluates diluted with ethanol, and concentrated to the volume of about 1 ml. The present products were compared with those obtained by the N,N′-dicyclohexylcarbodiimide polycondensation (for the results see Table I).

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Translated by J. Pliml.