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2,2,2-TRICHLOROETHYL 2-CHLOROPHENYL PHOSPHOROCHLORIDATE A CONVENIENT REAGENT FOR THE FORMATION OF INTERNUCLEOTIDE LINKAGES

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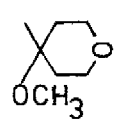
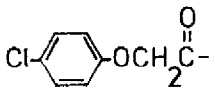
In the last few years, several methods[1-3] have been developed for the synthesis of oligonucleotides by the *modified* phosphotriester approach A common feature of all these methods is that a 5'-protected nucleoside (e g 1) is phosphorylated with a suitably-substituted phosphate (for example, 2,2,2-trichloroethyl phosphate[1] or aryl phosphates[2-3]) in the presence of TPS[4] ,followed by isolation of the phosphorylated product by column chromatography The thus obtained phosphodiester is then protected by another suitable protective group (for example, cyanoethyl[1-2] or 2,2,2-trichloroethyl[3]) and TPS[4] to give a phosphotriester (e g 3)
The latter derivative is a crucial intermediate in the *modified* triester approach, to be effective in the synthesis of oligonucleotides this derivative has to meet the following criteria firstly, it must be easily available, secondly, the conditions necessary for the removal of one of the protective groups from this intermediate (e g 3), to give a phosphodiester (e.g. 4), must be selective and may not involve time-consuming purification procedures
Up to now, none of the published methods[1-3] is in agreement with the above specified criteria
We now wish to report that the use of *2,2,2-trichloroethyl 2-chlorophenyl phosphorochloridate* 2 - necessary for the phosphorylation of a 5'-protected nucleoside (e g 1) - together with a *selective removal* of the 2,2,2-trichloroethyl group from the phosphotriester 3 with the reagent - *Zn/TPSOH/pyridine* - followed by *extraction* of the formed diester 4, represents an effective procedure for the introduction of internucleotide linkages
The latter will be demonstrated in the synthesis of the *hexanucleotide pTpTpTpTpT (10)*
Reagent 2 may be readily prepared by adding dropwise triethylamine (0 1 mole) to a stirred solution of 2-chlorophenyl phosphorodichloridate[5] and *2,2,2*-trichloroethanol (each, 0 1 mole) in dry ether Work-up of the reaction mixture and distillation gave 2[6] in 70% yield, b p 130-135°/0.15 mm
The protected nucleotide 3 (R = II) was obtained by reacting together 1[7] (R = II, 5 mmole) and 2 (6 mmole) in dry pyridine (30 ml) Work-up of the products after 16 hr and purification by Short Column Chromatography[8] gave 3[6], which was further isolated as a homogeneous (t l c) colourless solid in 90% yield
The formation of diester 4 may be regarded as the crucial step in the present work, it was performed by treating 3 (R = II, 2 5 mmole) in pyridine (20 ml) and 2,4,6-tri-isopropylbenzenesulphonic acid (TPSOH, 0 5 mmole) with Zn-dust (18 mmole) After 3 min excess Zn was removed by filtration and the filtrate - after dilution with $CHCL_3$ (80 ml) - was washed with triethylammonium bicarbonate (TEAB, 1 M, pH 7 5, 40 ml) The seperated organic layer was ren-



I



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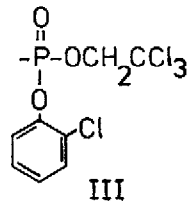

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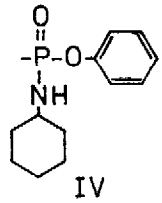
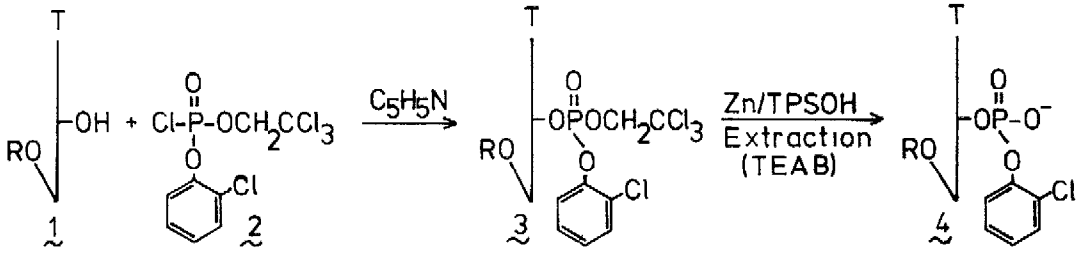
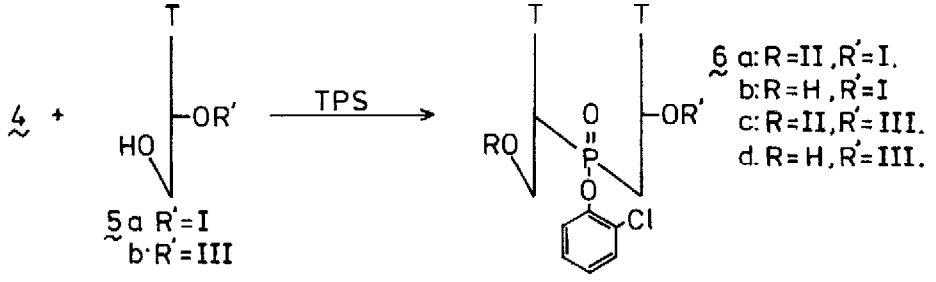
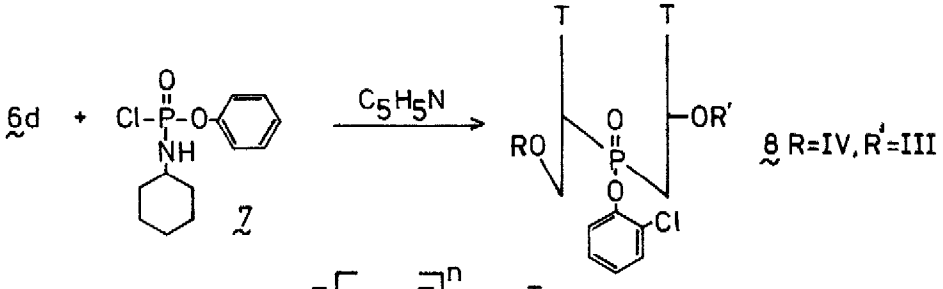
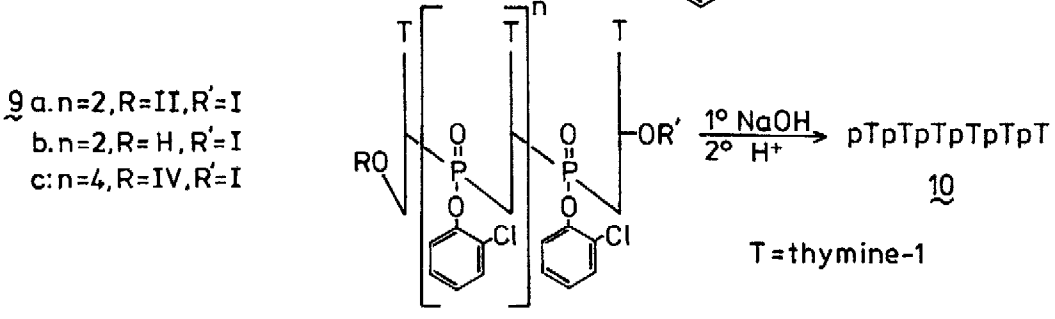


IV









T = thymine-1

dered anhydrous by repeated co-evaporation with pyridine to give a solution of 4 (R = II) in the same solvent. The latter solution of 4 in dry pyridine (8 ml) was reacted together with 5a[9] (2.2 mmole) and TPS (2.5 mmole). Work-up of the products after 24 hr gave crude 6a, which after alkaline de-blocking[10] - K_2CO_3/MeOH - and purification by Short Column Chromatography afforded 6b as a homogeneous (t.l.c.) colourless solid in 86% yield[11].

The presence of the appropriate internucleotide linkage in product 6b was proved as follows. Firstly, the 5'-hydroxyl group of 6b was tetrahydropyranylated[9] and the product obtained was then treated with base (0.125 N alkali) followed by acid (pH 2). Examination of crude TpT thus obtained showed it to be pure (paper electrophoresis, t.l.c. MN-cellulose, high pressure liquid chromatography) for more than 98%. Furthermore, it was completely degraded to the expected products (pT, T and Tp, T) by enzyme digestion with venom and spleen phosphodiesterase, respectively.

In the same way, starting from 3 (R = II, 5 mmole), TPSOH (1 mmole), Zn (27 mmole), 5b[6] (4.5 mmole, obtained from 3 (R = II) in 90% yield) and TPS (5 mmole), 6c was isolated as a homogeneous solid in 87% yield[11]. Repitition of the alkaline de-blocking process on 6c gave 6d as a chromatographically pure solid in 80% yield.

Phosphorylation of 6d (1.2 mmole) with 7[10] (1.5 mmole) in pyridine (15 ml) afforded the fully protected dimer 8 in 68% yield.

The tetramer 9a was then prepared from dimer 6c (1.9 mmole), Zn (10 mmole), TPSOH (0.35 mmole), TPS (1.8 mmole) and dimer 6b (1.7 mmole) in the manner as described above for 6a, it was isolated as a homogeneous solid in 70% yield[11]. Short alkaline treatment[10] of 9a gave chromatographically pure 9b in 80% yield.

Finally the fully-protected hexamer 9c was prepared from dimer 8 (0.4 mmole), Zn (4.0 mmole), TPSOH (0.4 mmole), tetramer 9b (0.4 mmole) and TPS (0.5 mmole). Work-up of the products after 48 hr and purification by Short Column Chromatography gave 9c, which was isolated as a homogeneous (t.l.c.) colourless solid in 70% yield.

In order to obtain the unprotected oligonucleotide pTpTpTpTpTpT (10), fully protected 9c was subjected first to alkaline (0.125 N alkali at 20°) and then to acidic (pH 2, 20°) hydrolysis. Crude 10 was purified on DEAE-Sephadex, $(pT)_6$ 10 accounted for 95% of the total absorbance units (266 nm) eluted from the column.

The homogeneity and identity of 10 was established by t.l.c. (MN-cellulose), high pressure liquid chromatography and paper electrophoresis, it was furthermore completely digested by (i) venom phosphodiesterase to pT, (ii) alkaline phosphatase to $(Tp)_5T$ and the latter by spleen phosphodiesterase to Tp and T.

In conclusion, the present procedure which consists of a one-step phosphorylation of a 5'-protected nucleoside (e.g. 1) with reagent 2 and selective removal of one protecting group from a now easily available phosphotriester function (e.g. 3), together with an efficient extraction step for the isolation of the formed diester (e.g. 4), promises to be of general use in the synthesis of oligonucleotides.

It remains to be seen if this method is also applicable to the synthesis of oligonucleotides containing the nucleosides dC, dA and dG[12].

ACKNOWLEDGEMENT

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FOOTNOTES AND REFERENCES

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12 Recently we were able to synthesize - by exactly the same procedure - the dimers dCpC and dApT in excellent yields