A New Ribo-oligonucleotide Block Synthesized by Phosphorylation with *p*-Chlorophenyl *N*-Phenylchlorophosphoramidate

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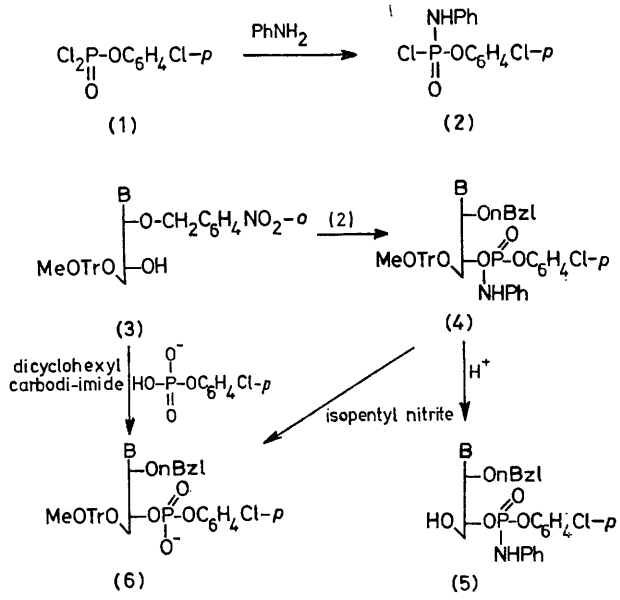
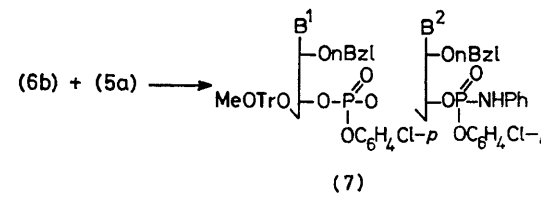
Summary Two types of nucleotides (**5**) and (**6**) have been synthesized as key intermediates for fully protected oligoribonucleotides which are suitable for elongation of the chain in the 3′- and 5′-directions by the triester method; condensation of these nucleotides yielded a fully protected dinucleotide [*e.g.* (**7**)] in high yield.

A NUMBER of phosphorylating reagents have been investigated for the preparation of phosphodiester intermediates in the phosphotriester oligonucleotide synthesis.[1–3] We have previously synthesized fully protected oligonucleotides with 3′-phosphorodianilidates[4] which gave 3′-phosphomonoesters by treatment with isopentyl nitrite.[5] We now report the synthesis of a new type of oligoribonucleotide block [*e.g.* (**7**)] which can be converted into a protected oligonucleotide with the 3′-phosphodiester suitable for the block condensation. For this approach the terminal units (**5a**—**d**) and internal units (**6a**—**d**) were synthesized as shown in the Scheme. Compounds (**3a**),[6] (**3b**,**c**),[7] and (**3d**)[8] were phosphorylated in pyridine with 1·5 equiv. of *p*-chlorophenyl *N*-phenylchlorophosphoramidate (**2**) (m.p. 145—149 °C) which was synthesized by treating (**1**) with aniline in benzene. Compound (**1**) was prepared by the method described for other phosphorylating reagents.[1] The intermediate (**4**) was treated with 80% acetic acid and the product (**5**) was isolated by chromatography on silica gel G. The yields of (**5a**), (**5b**), (**5c**), and (**5d**) were 73, 85, 75, and 54% respectively. Diastereoisomers of each nucleotide were separated and identified by elemental analysis, u.v. and n.m.r. spectroscopy, and paper chromatography of deblocked compounds. Compound (**5a**) could be crystallized from chloroform without chromatography. Recrystallization from methanol separated the diastereoisomers of (**5a**), the faster moving compound on t.l.c. having m.p. 228—229 °C, and the slower moving compound m.p. 115—118 °C.

The phosphodiesters (**6**) can be prepared from (**4**) by treatment with isopentyl nitrite but (**6a**—**d**) were synthesized more easily by condensation of (**3**) and *p*-chlorophenyl phosphate with dicyclohexylcarbodi-imide. Compounds (**6a**—**d**) were isolated by extraction with chloroform, in yields of 88—96%.



SCHEME. nBzl = $CH_2C_6H_4NO_2$-*o*
a; B = uracil-1-yl
b; B = N^4-benzoylcytosin-1-yl
c; B = N^6-benzoyladenin-9-yl
d; B = N^2-isobutyrylguanin-9-yl



B^1 = N^4-benzoylcytosin-1-yl
B^2 = uracil-1-yl

Condensation of the triethylammonium salt of (**6b**) (2·05 mmol) and (**5a**) (1·58 mmol) with mesitylenesulphonyl triazolide[3] (6·15 mmol) in pyridine at 30 °C for 36 h yielded the dinucleotide (**7**) (1·35 mmol, 85%) after chromatography

on silica gel G. A portion of (**7**) was deblocked for identification by treatment with isopentyl nitrite in pyridine–acetic acid (1 : 1) overnight, followed by concentrated ammonia at 55 °C for 3 h and 80% acetic acid at 30 °C for 2 h. C(nBzl)-U(nBzl) OC_6H_4Cl-*p* was isolated by paper chromatography (isopropyl alcohol–conc. ammonia–water, 7 : 1 : 2, v/v) and irradiated with u.v. light.[6,7] C-Up and C-U>p thus obtained were identified by enzymic hydrolyses with bacterial alkaline phosphatase and pancreatic RNase. Cp and U were obtained from C-U in the correct ratio. The dinucleotide (**7**) is a suitable intermediate for elongation of the chain in both directions.

(*Received, 14th June* 1978; *Com.* 628.)

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