SYNTHESIS OF DEOXYRIBOOLIGONUCLEOTIDES BY USING AROMATIC PHOSPHORAMIDATES AS THE PROTECTING GROUP FOR THE 3'-PHOSPHO ENDS

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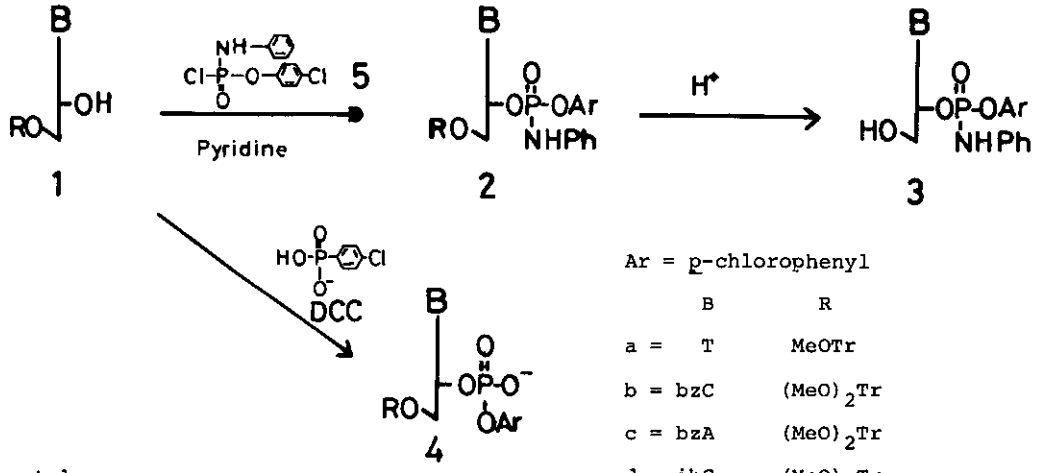
p-Chlorophenyl phosphoranilidochloridate was used to phosphorylate the 3'-hydroxyl groups of N,5'-O-protected deoxyribonucleosides. These nucleotides served as the 3'-terminal unit in the syntheses of some protected oligonucleotides which were used in a block condensation after removal of the anilido groups by treatment with isoamyl nitrite. Using this procedure, deoxyribooligonucleotides e. g. a decanucleotide containing a restriction endonuclease recognition sequence were synthesized.

Aromatic phosphoramidates have previously been used as protecting groups for phosphomono[1]- and phosphodi[2]-esters in syntheses of ribooligonucleotides. p-Chlorophenylphosphoranilido chloridate[3] was reacted with the 3'-hydroxyl group of protected ribonucleosides and these nucleotides served as the 3'-terminal unit in ribooligonucleotide syntheses by the phosphotriester approach.[2] Recently, several groups have published improved phosphotriester methods for the synthesis of deoxyribooligonucleotides using a variety of combinations of protecting groups for phosphates and 5-hydroxyl groups.[4-7] In this paper we report that deoxyribooligonucleotide blocks have been synthesized by the phosphotriester method using an aromatic phosphoramidate as the protecting group for the 3'-termini and these blocks have been condensed to yield polynucleotides with defined sequences.

Chart 1 illustrates the phosphorylation of 5'-O-monomethoxytritylthymidine[8] (1a), 5'-O-dimethoxytrityl-N-benzoyldeoxycytidine[8] (1b), 5'-O-dimethoxytrityl-N-benzoyldeoxyadenosine[8] (1c) and 5'-O-dimethoxytrityl-N-isobutyryldeoxyguanosine[9] (1d) with p-chlorophenylphosphoranilido chloridate[3] (5). The fully protected nucleotides (2) were obtained using the conditions summarized in Table I. The deoxycytidine derivative(1b) was phosphorylated at low temperature to avoid side reactions which presumably involved 2,3'-O-cyclonucleoside formation. The 5'-protecting group was removed by acidic treatment with benzenesulfonic acid at 0°[9b]



Ar = p-chlorophenyl

	B	R
a =	T	MeOTr
b =	bzC	$(MeO)_2Tr$
c =	bzA	$(MeO)_2Tr$
d =	ibG	$(MeO)_2Tr$

Chart 1

Table I Reaction Conditions for Phosphorylation of 1

Nucleoside (mmol)	Reagent (5) (mmol)	Temperature	Time (hr)	Overall yield of 3 (%)
1a	8	12	room temperature	48	87
1b	0.5	1.1	-20°	46	56
1c	2.6	5.0	room temperature	20	84
1d	0.5	1.2	room temperature	16	64

except for 1a (for which 80% acetic acid was used). The nucleotides (3) were isolated by chromatography on silica gel. The phosphodiesters (4) were prepared by phosphorylation of N,5'-O-protected nucleosides (1) using p-chlorophenyl phosphate plus dicyclohexylcarbodiimide and were used as intermediates for the synthesis of oligonucleotide blocks.

Chart 2 shows a synthesis of a hexanucleotide formed by the condensation of blocks prepared from these mononucleotide units. 3a and 4a were reacted using a 3-fold excess of mesitylenesulfonyl triazolide[10] (MST) as the condensing reagent. The fully-protected dinucleotide (6) was isolated by chromatography on a column of silica gel in a yield of 58% and the 5'-monomethoxytrityl group was removed by treatment with 80% acetic acid to elongate the block in the 5'-direction. 7 was then treated overnight with a slight excess of 5'-O-monomethoxytrityl-N-isobutyryl-deoxyguanosine 3'-p-chlorophenylphosphate (8) using a 3-fold excess of MST with respect to 8. The protected trinucleotide (9) was isolated similarly in a yield of

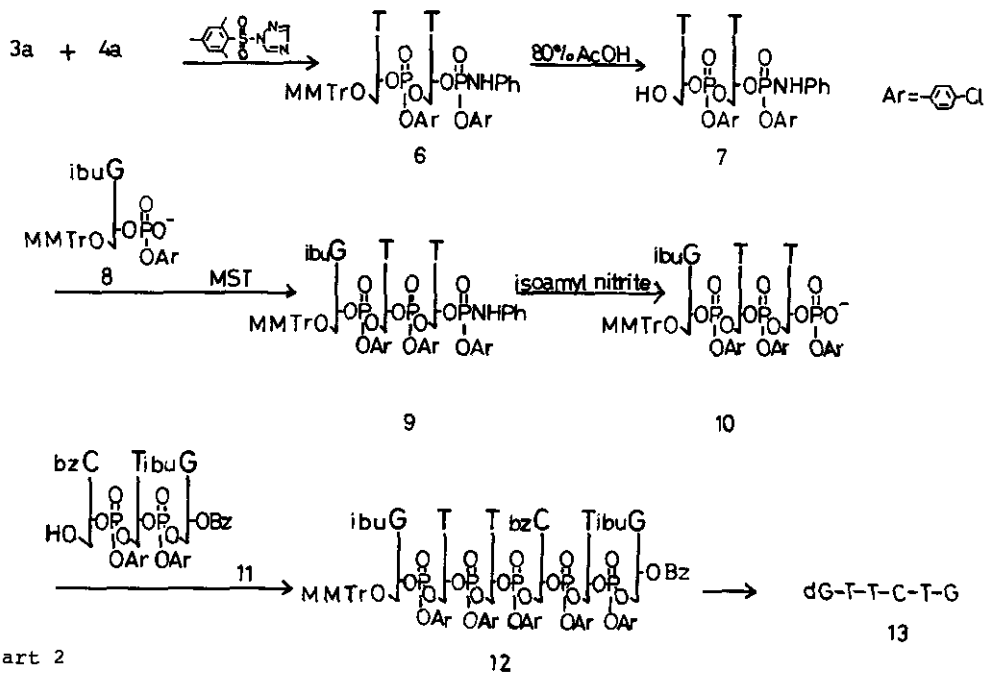


Chart 2

54% and treated with isoamyl nitrite in 1:1 pyridine-acetic acid to yield the compound having a 3'-phosphodiester end (10) ready for the condensation with nucleotide blocks having the 5'-hydroxyl group free (e. g. 11). 11 was prepared from 3'-O-benzoyl-N-isobutyryldeoxyguanosine[5] by successive condensation with 4a and 4b using MST. The mono- or dimethoxytrityl group was removed by treatment with 2% benzenesulfonic acid at 0°. As shown in Chart 2, 10 (0.30 mmol) and 11 (0.20 mmol) were condensed with mesitylenesulfonyl 4-nitro-imidazolide[5] (0.9 mmol) at 30° for 36 hr to yield the protected hexamer (12, 0.14 mmol). Deblocking of 12 was performed by treatment with 4:1 concentrated ammonia-pyridine at 50° for 4.5 hr followed by 80% acetic acid at 30° for 1.5 hr. The deblocked hexamer dGTTCTG was characterized as described for the ribooligonucleotides.[2] The protected hexanucleotide (12) could be elongated in the 5'-direction by condensation with oligonucleotides having the 3'-phosphodiester group (e. g. 10) after removal of the monomethoxytrityl group.

A decanucleotide dCCCTGCAGGG containing the recognition sequence for a restriction endonuclease Pst (dCTGCAG) was synthesized similarly. Other deoxyoligonucleotides which are complementary to sequences found in viral RNA's[11] have also

been synthesized. p-Anisidine and o-chlorophenol could be used in the synthesis of phosphorylating reagents analogous to 5. Results obtained in condensations involving nucleotides prepared by these reagents will be reported elsewhere.

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