

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 N₅'-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 phosphomono¹- and phosphodi²-esters in syntheses of ribooligonucleotides. p-Chlorophenylphosphoranilido chloridate³ 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-monomethoxytritylthymidine⁸ (1a), 5'-O-dimethoxytrityl-N-benzoyldeoxycytidine⁸ (1b), 5'-O-dimethoxytrityl-N-benzoyldeoxyadenosine⁸ (1c) and 5'-O-dimethoxytrityl-N-isobutyryldeoxyguanosine⁹ (1d) with p-chlorophenylphosphoranilido chloridate³ (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}

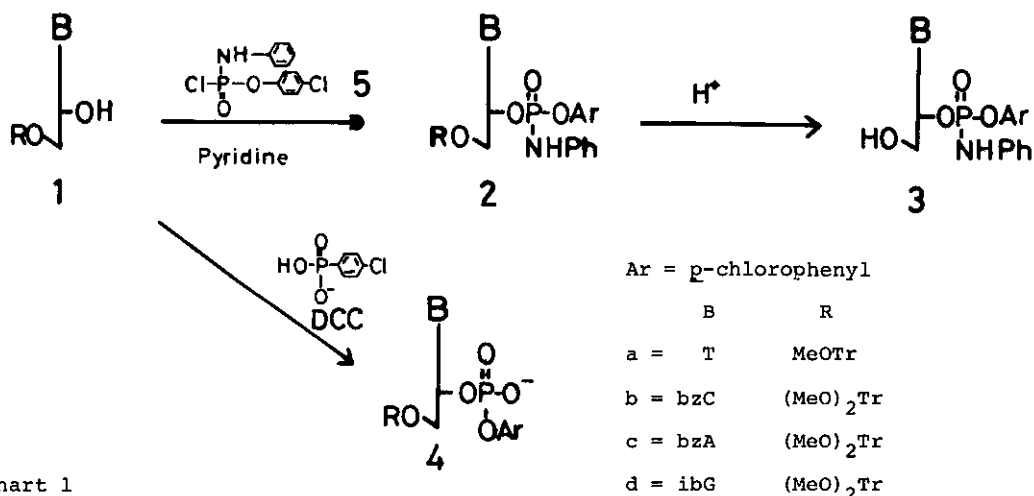


Chart 1

Table I Reaction Conditions for Phosphorylation of 1

Nucleoside (mmol)	Reagent (5) (mmol)	Temperature	Time (hr)	Overall yield of 3 (%)	
<u>1a</u>	8	12	room temperature	48	87
<u>1b</u>	0.5	1.1	-20°	46	56
<u>1c</u>	2.6	5.0	room temperature	20	84
<u>1d</u>	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 phosphodiester (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 triazolid¹⁰ (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

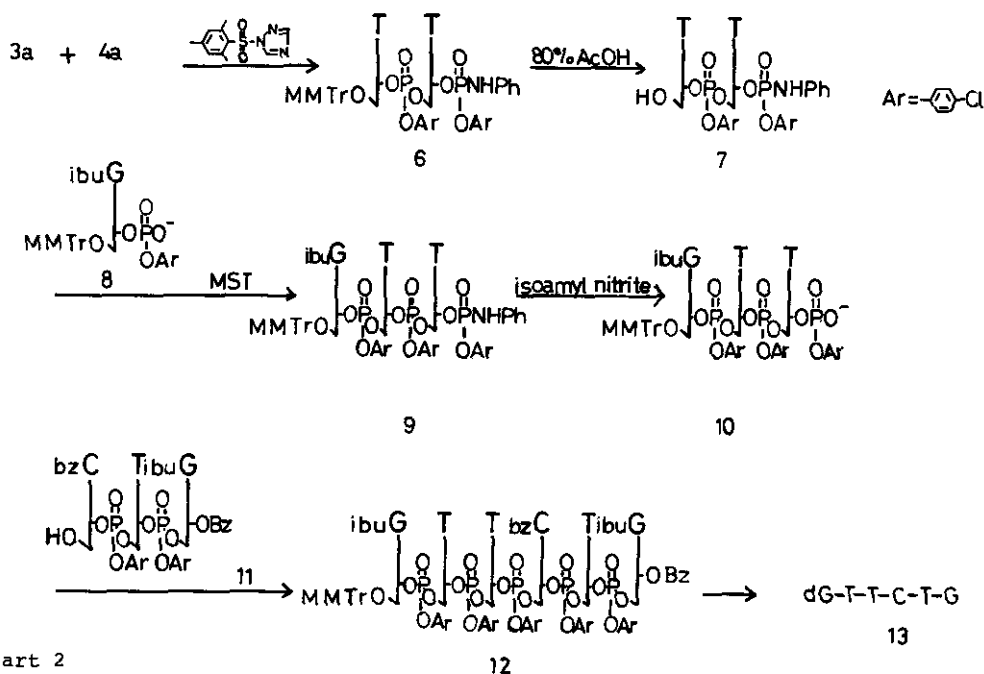


Chart 2

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-isobutyryldeoxyguanosine⁵ 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-nitroimidazole⁵ (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's¹¹ 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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