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A NEW METHOD FOR THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES CONTAINING INTERNUCLEOTIDE PHOSPHORAMIDATE BONDS

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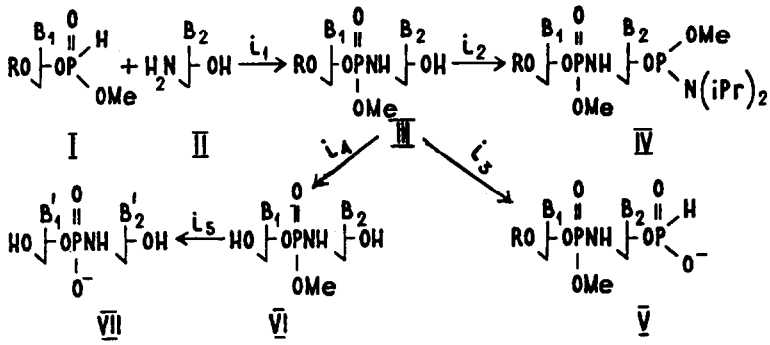
Abstract. Condensation of 5'-O- and N-blocked deoxynucleoside 3'-O-methyl-H-phosphonates (I) with 5'-amino-2',5'-dideoxynucleosides (II) in the presence of CCl_4 and Et_3N produces dinucleoside phosphoramidates (III) which were used in oligonucleotide synthesis by phosphoramidite or H-phosphonate methods.

Oligonucleotides containing phosphoramidate internucleotides bonds are of interest as analogs of biologically-important phosphates. Several methods have been described for synthesis of such compounds in aqueous media.[1-4] However, the small phosphoramidate oligonucleotide analogs thus obtained cannot be incorporated into the oligonucleotide chain, since they lack the blocking groups conventional for oligonucleotide chemical synthesis and ensuring selective coupling and solubility in organic media. As to the synthesis of such compounds in organic media, it has not been commonly used as yet [5,6].

In this work we propose a new efficient method of obtaining oligodeoxyribonucleotides containing P3'→N5' phosphoramidate internucleotide bonds, which is based on the Atherton-Todd reaction of oxidative phosphorylation of amines [7,8] (Scheme).

The 5'-protected nucleoside 3'-O-methyl-H-phosphonates (I) were synthesized as described [8]. 5'-Aminonucleosides (II) were derived by reduction of 5'-azidonucleosides obtained by the single-step technique [9]. Besides, it should be noted that substantial amounts of (I) are formed as a byproduct in the phosphoramidite method of oligonucleotide synthesis, upon hydrolysis of tetrazole-activated nucleoside 3'-O-(N,N'-dialkylamido) methylphosphites commonly used in 10-15-fold excess[10].

SCHEME



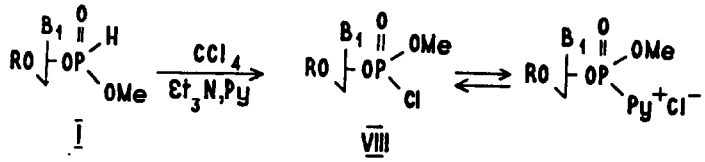
B_1=T, C^{bz}, A^{bz}, G^{ibu}; B_2=T, C^{bz}; B_1'=T, C, A, G; B_2'=T, C;

R = MeOTr, $(MeO)_2$Tr;

i_1= CCl_4, Et_3N; i_2= $MeOP[N(iPr)_2]_2$; i_3= $PTri_3$;

i_4= $ZnBr_2$ or CCl_3COOH or CF_3COOH; i_5= $PhSEt_3NH$.

Condensation of (I) and (II) was carried out in pyridine [11], whereby the reaction was complete in less than 2 min with a phosphoramidate (III) yield of 95-98%, while in dimethylformamide the reaction takes more than 2 h and the end-product yield is 75-80%. Pyridine appears to act as a nucleophilic catalyst in this reaction, and (I) converts into the highly reactive compound (VIII)[12]:



The structure of compounds (VII) obtained by deblocking (III) was confirmed by acid hydrolysis (80% CH_3COOH, 5 min at 50°C), resistance to snake venom phosphodiesterase which does not cleave the phosphoramidate bonds [13], and ^{31}P NMR spectroscopy. Compounds (III) were isolated by silica gel column chromatography. Phosphoramidates are known to be acid-labile [5,6,14], and it was therefore necessary to determine the stability of the phosphoramidate diester group in (III) with respect to the protic agents used in stepwise detritylation. For this purpose, the dinucleoside phosphoramidate (VI) (B_1=B_2=T) obtained according to the scheme given above[8] was treated with 0.9% (0.1 M) CF_3COOH or 3% CCl_3COOH in methylene chloride for 30 min. The phosphoramidate

diester group of compound (VI) proved stable under such conditions as revealed by reversed-phase HPLC and TLC. This appears the reason for the stability is the $n_N \longrightarrow \pi^*_{P=O}$ conjugation of the nitrogen free electron pair with phosphoryl group[15]. The phosphoramidate monoesters lacking this conjugation are much more acid labile than phophoramidate diesters. Similarly, the phosphoramidate diester group has been shown to withstand the oxidizing and the capping reagents used in the oligonucleotide synthesis.

The dinucleotide phosphoramidate (III, B_1=B_2=T) was converted into phosphoramidite (IV) and H-phosphonate (V) components by the known way for the synthesis of oligonucleotides IX-XII with phosphoramidate internucleotide bonds by phosphoramidite [10,16] and H-phosphonate [17] methods. The efficiency of internucleotide bond formation in the reaction of nucleotide component (IV) with nucleoside component, immobilised on the polymer support in the standard conditions reaches 95-98% and for component (V) - 94-95%. After cleavage from the solid support and removal of all protecting groups the oligonucleotides IX-XII were purified by 20% PAAG electrophoresis.

5' TTGCCAATT$_{pN}$TGGCAA (IX) 5' AAGCCAAAT$_{pN}$TGGCAA (X)

5' TTGCCAAT$_{pN}$TTGGCTT (XI) 5' $(T_{pN}T)_{20}T$ (XII)

pN = P3'⟶N5 internucleotide bond

Their homogeneity were confirmed by ion-exchange and reversed-phase HPLC. The presence of the phosphoramidate monoester bond in the oligonucleotides was confirmed by selective hydrolysis with 80% acetic acid (Fig.)[5,6].

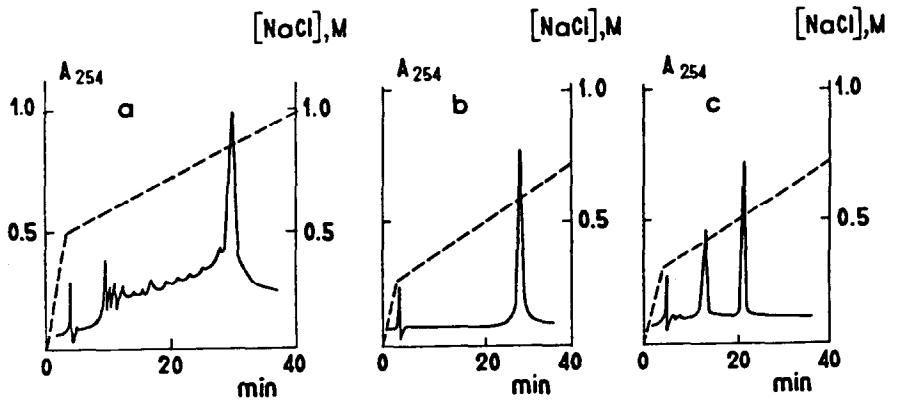


Fig. Ion-exchange FPLC: a)reaction mixture synthesis of the $(Tp_NT)_{20}T$ by H-phosphonate method; b)pentadecanucleotide (IX), purified by PAAG electrophoresis; c)hydrolyzate of 80% acetic acid, 30 h, 25°C, oligonucleotide (IX); column MonoQ 5/5, Pharmacia, gradient NaCl in 0.01M NaOH, pH 12.

Thus, our results allow a conclusion that this method can be used to obtain oligonucleotides with P3' → N5' internucleotide bonds. The high yields, absence of by-products, and experimental simplicity make this method particularly attractive.

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