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SOLID-PHASE OLIGONUCLEOTIDE SYNTHESIS WITH AFFINITY-CHROMATOGRAPHIC SEPARATION OF THE PRODUCT [1]

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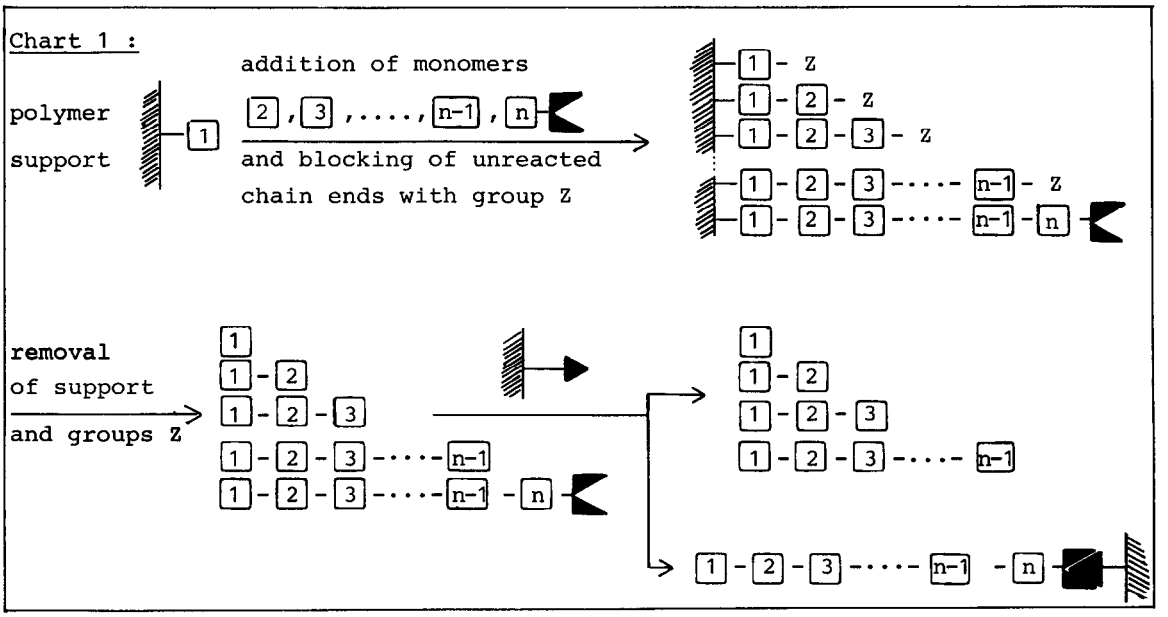
INTRODUCTION : The solid-phase approach to biopolymer synthesis has the disadvantage that the support is a "polyfunctional" blocking group. This fact essentially necessitates a synchronous growth of the grafted chains. If this cannot be achieved the product released from the support contains the desired sequence to-gether with homologous and truncated compounds. The difficulties encountered in separating this mixture may limit the applicability of the support synthesis. This is the situation in the preparation of oligonucleotides, where the yields of internucleotide bond formation are generally far from quantitative.

In recent work we have used affinity blocking groups in the separation of products from nucleotide co-condensations [2]. Such groups had previously been applied for the terminal blocking of support-bound oligonucleotides [3], and we have now used their potential to single out the desired sequences from solid-phase synthesis products. The general reaction scheme is shown in Chart 1 . Two examples for the application of this synthesis scheme are described in this

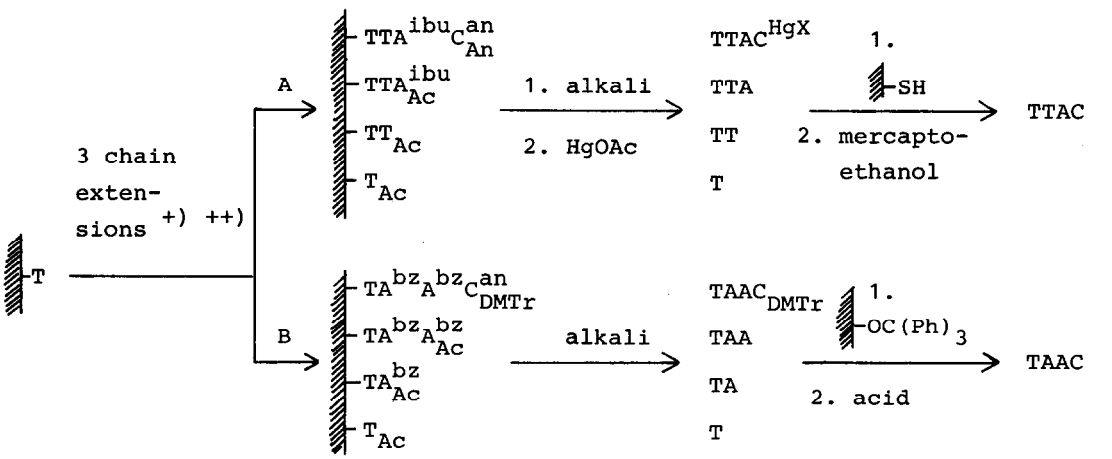


paper. While this work was in progress [4] a similar procedure for polypeptide synthesis was reported by D.E. Krieger et al.[5] .

RESULTS : We followed two pathways, described in Chart 2 as A and B, in the preparation of the tetranucleoside triphosphates dTTAC resp. dTAAC . As a support commercial Bio Rex 70 [R] , converted to the 2-chlorocarboxypropyl ester [6] , was grafted with (A) 0.3 resp. (B) 0.5 mmol deoxythymidine / g carrier. After 3 chain elongation cycles, each including reaction with an appropriate blocked nucleotide (the monomers shown in Chart 1 and Table 1 were applied in 10-fold excess; TPS, 3-fold over nucleotides; solvent pyridine; 30 h at 50°), acetylation and (except last cycle) treatment with 0.1 M p-toluenesulfonic acid in acetonitrile [7] , the product was removed from the carrier by alkali.

A part of the released material was separated by DEAE cellulose chromatography using an ammonium bicarbonate gradient. Alternatively, vis-spectroscopy of the acid solutions obtained on removal of the terminal monomethoxytrityl- (470 nm) resp. dimethoxytrityl (494 nm) residues permits the analysis of extent of internucleotide bond formation. The products and yields of chain elongation are listed in Table 1 . The residual material in approach A was treated with mercuric acetate in sodium acetate buffer, pH 6 [8] and applied to a column of sulfhydryl cellulose [9]. After eluting all non-mercurated material with aqueous buffer (0.1 M sodium acetate / 0.5 M sodium chloride, pH 6) the admixture of 0.1 M mercaptoethanol [8] yielded a second fraction containing

Chart 2 :



+) monomers: (A) pdT_{MMTr}, pdA^{ibu}_{DMTr}, pdC^{an}_{An}; (B) pdA^{bz}_{DMTr}, pdA^{bz}_{DMTr}, pdC^{an}_{DMTr}

++) chain extension = 1. reaction with blocked nucleotide; 2. acetylation; 3. acid treatment (steps 2 and 3 omitted in extension no. 3).

Table 1 :

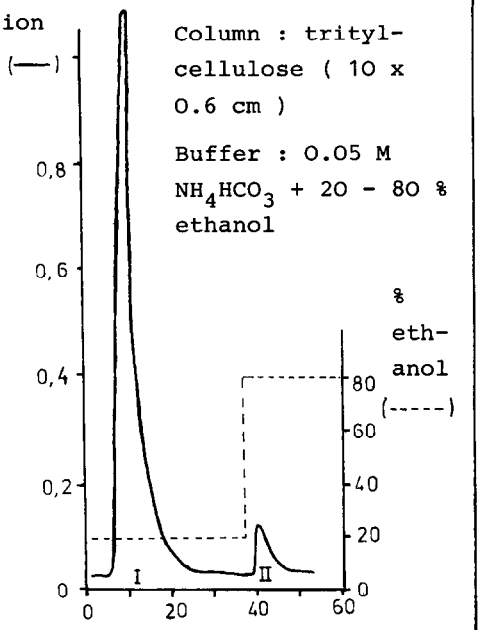
Chain Extension no.	Nucleotide Reactant	Product	Yield (%) +)++)	Product Composition (%) ++)	+++)
-	-	dT	-	67	
A 1	pdT_{MMTr}	dT-T	18	13	94
A 2	pdA^{ibu}_{DMTr}	dT-T-A	33	13	
A 3	pdC^{an}_{An}	dT-T-A-C	27	7	6
-	-	dT	-	71	
B 1	pdA^{bz}_{DMTr}	dT-A	8	4	87
B 2	pdA^{bz}_{DMTr}	dT-A-A	77	9	
B 3	pdC^{an}_{DMTr}	dT-A-A-C	61	16	13

+) Yield per elongation, Mol-%

++) Composition determined from DEAE-chromatography and/or spectroscopy (% $O.D._{260}$)

+++) Composition determined from affinity chromatography (% $O.D._{260\ nm}$)

Fig. 1: Affinity-chromatographic Separation of $dTAAC_{DMTr}$ from By-products of Support Synthesis (total 70 $O.D._{260\ nm}$)



exclusively and quantitatively the desired sequence dTTAC (compare Table 1; the -HgX - group is removed in the elution buffer [8]). In approach B the support synthesis product was directly chromatographed on trityl cellulose [2], and the elution profile shown in Fig. 1 was obtained. Peak I, collected on washing with 0.05 M ammonium bicarbonate + 20 % ethanol, contained all non-tritylated material. On raising the ethanol content to 80 % a second peak was eluted, which, after detritylation, was found to be the expected tetramer dTAAC, the yield being in good accordance with the previous determination (Tab. 1). Both tetranucleoside triphosphates were pure by chromatographic standards, and the sequence was confirmed by enzymatic and base ratio analysis (dA : dC : dT = 1.1 : 1.0 : 2.1 (A) resp. 2.0 : 0.9 : 1.0 (B)).

DISCUSSION : The introduction of terminal affinity blocking groups, which, according to our studies, permits the removal rapidly and selectively, of the desired sequence from a solid-phase oligonucleotide product, destinctly improves the efficiency of the support method and may be expected to extend its range of applicability to longer chains than hitherto synthesized. Although good yields of internucleotide bond formation are still desirable (and, in our case , seem to be partially precluded by a spacer problem which is under investigation), they are now not a prerequisite for the successful application

of the solid-phase technique, as long as the unreacted shorter chain termini can be quantitatively masked. The latter, obviously, is the case in our experiments. Two features of our new approaches to oligonucleotide support synthesis should be further emphasized : 1. Any given oligonucleotide sequence can be prepared using one of the alternative terminal affinity blocking procedures, and 2. the use of tritylated monomer intermediates provides for a ready means of reaction control. The application of these new approaches to the preparation of longer oligonucleotide chains of biological interest is the aim of further work done in our laboratory.

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