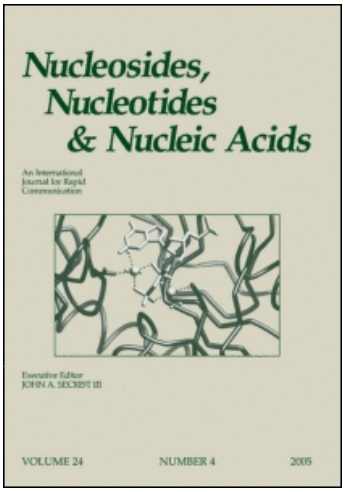

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A New Option in Solid Phase Synthesis of DNA-Fragments

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A NEW OPTION IN SOLID PHASE SYNTHESIS OF DNA-FRAGMENTS

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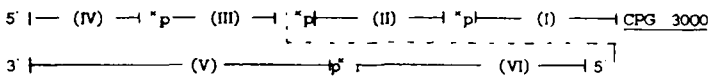
Abstract: The use of a new support suitable for enzymatic conversion of the immobilized oligonucleotide chain and the possibility of post-synthesis deprotection without release from the polymer is described.

The application of immobilized oligonucleotides as hybridization probes or their use for the isolation of a target DNA sequence requires principally a DNA strand devoid of any protecting groups. The current scheme of oligonucleotide synthesis involves the cleavage of the polymer support concommitantly with the deprotection of the nucleobases. Immobilization of unprotected oligonucleotides has been achieved hitherto in general by binding a previously prepared sequence from solution. We have recently shown[1] that CPG 3000, a support material with wide regular pores, allows chemical as well as enzymatic elongation of oligothymidylates. In order to extend this strategy to oligonucleotides consisting of all four bases, it was necessary to find a selective reagent which is capable of removing the exocyclic nitrogen and phosphate protecting groups without cleavage from the support.

Ethanolamine, diethyleneamine or dibutyleneamine were found to be suitable reagents to deblock usually ptotected nucleotides. Kinetic studies showed that the ß-cyanoethyl group is removed at r.t. within two minutes, whereas the cleavage of the benzoyl and isobutyryl base protecting groups require up to two hours. This is in accord with the earlier findings of Letsinger et. al.[2], who showed that it was possible to remove N-benzoyl-groups from nucleobases with a mixture of hydrazine hydrate, pyridine and acetic acid without concurrent hydrolysis of ester bonds. In order to prove the applicability of these reagents to a combined chemical and enzymatic synthesis, we assembled sequence 1 of a ß-endorphine gene (Scheme 1) on CPG 3000 according to the usual phosphoramidite approach.[3] After removal of the dimethoxy- trityl group, the support material was treated with ethanolamine for two hours at r.t., filtered off and washed several times with acetonitrile, water and ether. Sequence 2 - 6 were synthesized as above with CPG 1400, fully



sequ. 1: 5' pCGT ACT ACC CTC GAT GAA TTC TTT TT – CPG 3000
sequ. 2: 5' pGCG TCT GGC TCT TCT CGG AGG TCA TGA AAC CGC
sequ. 3: 5' pTCT TGA TGA TGG CGT TTT TGA ACA GCG TCA CCA GGG
sequ. 4 5' TT GCT GCA GTT ATC ACT CGC CCT TCT TGT AGG CGT
sequ. 5: 3' AA CGA CGT CAA TAG TGA GCG GGA AGA ACA TCC GCA AGA ACT ACT ACC GCp
sequ. 6: 3' rA AAA ACT TGT CGC AGT GGT CCC CGC AGA CCG AGA AGA GCC TCC AGT ACT TTG GCG GCA TGG ATG GGA GCT ACT TAA GAA

Scheme 1:

Figure 1:

Synthesis of a gene for ß-endorphine by DNA ligase reaction on CPG 3000 and in solution

lane 1: solid phase kinase reaction of sequence 1 (100 µg support, 0.1 µM γ-^{32}p-ATP, 0.1 mM ATP, 40 mM Tris.Cl pH 7.6, 10 mM $MgCl_2$, 1 mM DTT, 5% glycerine, 4u T4 PN kinase)

lane 2: enzymatic synthesis of ß-endorphine on CPG 3000 (100 µg support, 600 pmol of fragments 2-6, 76 mM Tris.Cl pH 7.6, 150 µg ATP, 1mM $MgCl_2$, 6 u DNA ligase)

lane 3: DNA ligase reaction in solution without fragment 1 according to Scheme 1.

deprotected and purified by gel electrophoresis. After radioactive labelling and phosphorylation of the still immobilized sequence 1 we followed the conventional combination of sequentially overlapping oligomers with T4 DNA ligase for the construction of a gene for ß-endorphine.[4] At all stages of the synthesis the product was characterized after cleavage from the support (ammonia, r.t.) by gel electrophoresis (see Fig. 1).
This trategy may provide gene synthesis by combined chemical and enzymatic reactions on a solid support.

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