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IMPROVED SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES

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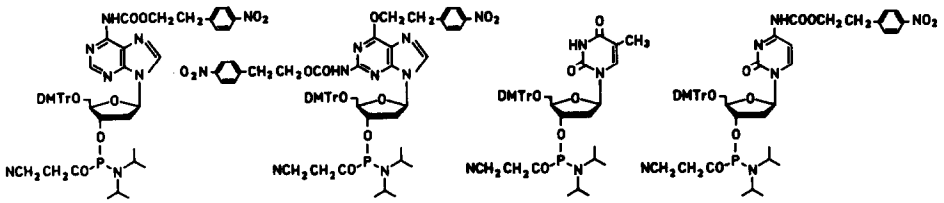
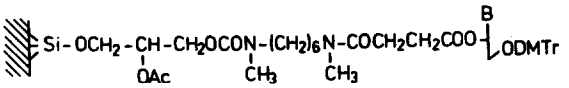
Abstract. The design of a new polymer support in combination with the well experienced β-eliminating protecting groups offers an improved approach for automated oligonucleotide synthesis. The advantages are notorious by the possibility of preparing fully deblocked still support-bound oligomers, which result on final liberation in high yield, easy isolation and high purity. This approach also reveals some options, which have so far not been realized in the field of solid phase synthesis of nucleic acid fragments.

The development of the phosphoramidite methodology[1,2] can be regarded as a break-through in chemical solid phase supported oligodeoxyribonucleotide synthesis due to a high degree of automatisation of the chain elongation process. A great number of variations of the original method has been published, dealing mainly with changes in the blocking group patterns of the aglycone, sugar and phosphite moiety of the monomeric building blocks as well as alterations of the material of the solid matrix and the design of the spacer connecting the starting nucleoside covalently with the support. The excellent results obtained on application of the p-nitrophenylethyl (NPE) and p-nitrophenylethoxycarbonyl (NPEOC) blocking groups[3] in solution synthesis of oligonucleotides[4,5] prompted us to develop a solid phase synthesis strategy, in which all synthetic as well as the deprotection steps could be done while the oligonucleotide is still attached to the support allowing an easy removal of any by-products and excess of reagents by simple washing and filtration[6].
The new strategy afforded in the first place the synthesis of a new set of 3'-(2-cyanoethyl)-N,N-diisopropylamido-phosphites differing from the commonly used and commercially available building blocks by applying the p-nitrophenylethoxycarbonyl (NPEOC) group for amino protection instead of the benzoyl and isobutyryl group respectively and the additional blocking of the amide function of 2'-deoxyguanosine by the p-nitrophenylethyl (NPE) residue.

The syntheses have been achieved in the usual manner by protecting first the aglycone moiety[3] followed by 5'-O-dimethoxytritylation and subsequent phosphitylation with 2-cyanoethyl-



bis(N,N-diisopropylamino)-phosphane[7] under tetrazole catalysis. Furthermore a new type of modified solid support material had to be prepared since the ester function of the succinyl spacer of the commercially available LCAA-CGP supports is not stable under the deprotection conditions using DBU or MTBD in aprotic solvents. In order to overcome the intramolecular nucleophilic attack of the neighbouring deprotonated amide group[8,9] dihydroxypropyl-CPG (500A and 1400A pores) beads were reacted first with N,N'-carbonyldiimidazole and then with 1,6-bis-methylaminohexane as an aliphatic secondary amine spacer. Final coupling with the appropriately protected 2'-deoxynucleoside-3'-O-succinates and subsequent capping of the free hydroxyl groups of the matrix led to loadings of the anchored starting nucleoside of 20 micromoles per gram support.

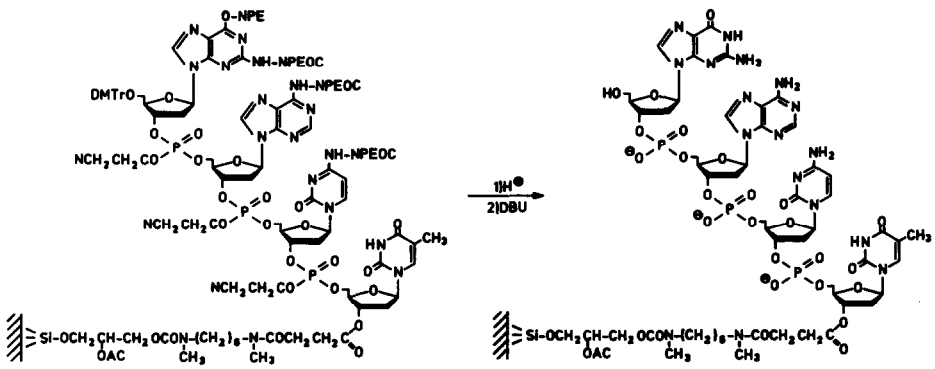


This support was proved to be completely stable under the deblocking conditions used for the NPE and NPEOC groups, while cleavage from the matrix can be achieved normally under hydrolytic conditions in conc. ammonia solution in less than two hours.

Several oligodeoxyribonucleotides have been synthesized in a DNA-synthesizer (Applied Biosystem Model 380 B) applying both acyl and NPE/NPEOC protected phosphoramidites for comparative studies. The chain elongation cycle was only slightly modified in using a 25 fold excess of the phosphoramidite reactant in regard to the support-bound 5'-OH component and a total condensation time of 60 seconds. The yield of each coupling step was determined by the colorimetric assay of the released dimethoxytrityl cation. The obtained yields indicate that the NPE/NPEOC protected phosphoramidites give similar or even better results as the common acyl blocked analogues.

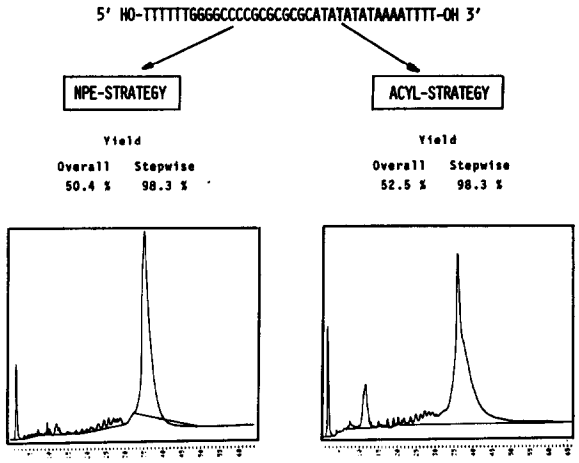
Tab. 1 - Results of comparative DNA-Syntheses

Sequence	Acyl-Strategy Overall %	Acyl-Strategy Stepwise %	NPE/NPEOC Strategy Overall %	NPE/NPEOC Strategy Stepwise %
HO-TTTTTTGGGGCCCCCGCGCGCGATATATATAAAATTTTTT-OH	52.5	98.3	50.4	98.2
HO-TAAAAACCCCGGGGCGCGCGCGTATATATATTTTAAAAAT-OH	53.4	98.4	61.0	98.9
HO-TTTTTTGGGGCCCCCGCGCGCGATATATATAAAATTTTTT) HO-TAAAAATTTTATATATATGCGCGCGCGGGGCCCCAAAAAT			48.6	99.8
HO-ATGCATGCGGGGTTTTATGCGCATAAAACCCCGCATGCAT-OH			55.6	98.3



The big advantage of the NPE/NPEOC-strategy became obvious during the deprotection procedure. Total deblocking of a synthesized oligonucleotide was achieved first by removal of the 5'-O-dimethoxytrityl group automatically by choosing the option "trityl off" in the synthesis program. The cyanoethyl as well as the NPE and NPEOC groups were then eliminated by treatment with 0.5 M DBU in acetonitrile during 6 hours at room temp. on the synthesizer. This way it is possible to obtain totally deprotected oligonucleotides, which are still attached to the solid support in high yields, demonstrating the superiority of this approach over the method of Seliger[10]. So far only less than 5 % of the oligonucleotide chain from the support was detected under the deprotection conditions on applying the "trityl on" program. Cleavage of the support-bound oligonucleotide finally was achieved by conc. aqueous ammonia by the common automated procedure to yield the crude product free of any by-products of the previous synthesis/deprotection steps. This material was then analysed by anion ex-

change HPLC on a Nucleogen 60-7 DEAE column[11] using a LiCl gradient (0-1 M) and compared with the same sequence material from the acyl strategy.



The improvements of this new approach have to be seen in a time-saving easy isolation procedure, better yields and higher purities of the oligonucleotides. Furthermore oligonucleotides containing modified base moieties will also be available on these lines.

REFERENCES

1) M.A. Dorman, S.A. Noble, L.J. McBride, M.H. Caruthers, Tetrahedron 40, 95 (1984).
2) H. Köster, J. Biernat, J. McManus, A. Wolter, A. Stumpe, Ch.K. Narang, N.D. Sinha, Tetrahedron 40, 103 (1984).
3) F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, Tetrahedron 40, 59 (1984).
4) R. Charubala, E. Uhlmann, F. Himmelsbach, W. Pfleiderer, Helv.Chim. Acta 70, 2028, (1987).
5) P.F. Torrence, D. Brozda, D. Alster, R. Charubala, W. Pfleiderer, J.Biol.Chem. 263, 1131 (1988).
6) K.P. Stengele, W. Pfleiderer, Nucleic Acids Res. Symp.Ser. No. 21, 101 (1989).
7) A.D. Barone, J.Y. Tang, M.H. Caruthers, Nucleic Acids Res. 12, 4051 (1984).
8) T. Reiner, Ph.D. Thesis Universität Konstanz, 1988.
9) T. Brown, C.E. Pritchard, G. Turner, S. Salisbury, J.Chem.Soc. Chem.Commun. 1989, 891.
10) S. Berner, G. Gröger, K. Mühlegger, H. Seliger, Nucleosides & Nucleotides 8, 1165 (1989).
11) Macherey-Nagel, D-5160 Düren / West Germany.

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