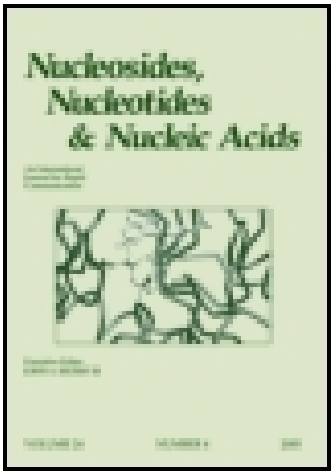

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An Inverse Approach in Oligodeoxyribonucleotide Synthesis

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AN INVERSE APPROACH IN OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

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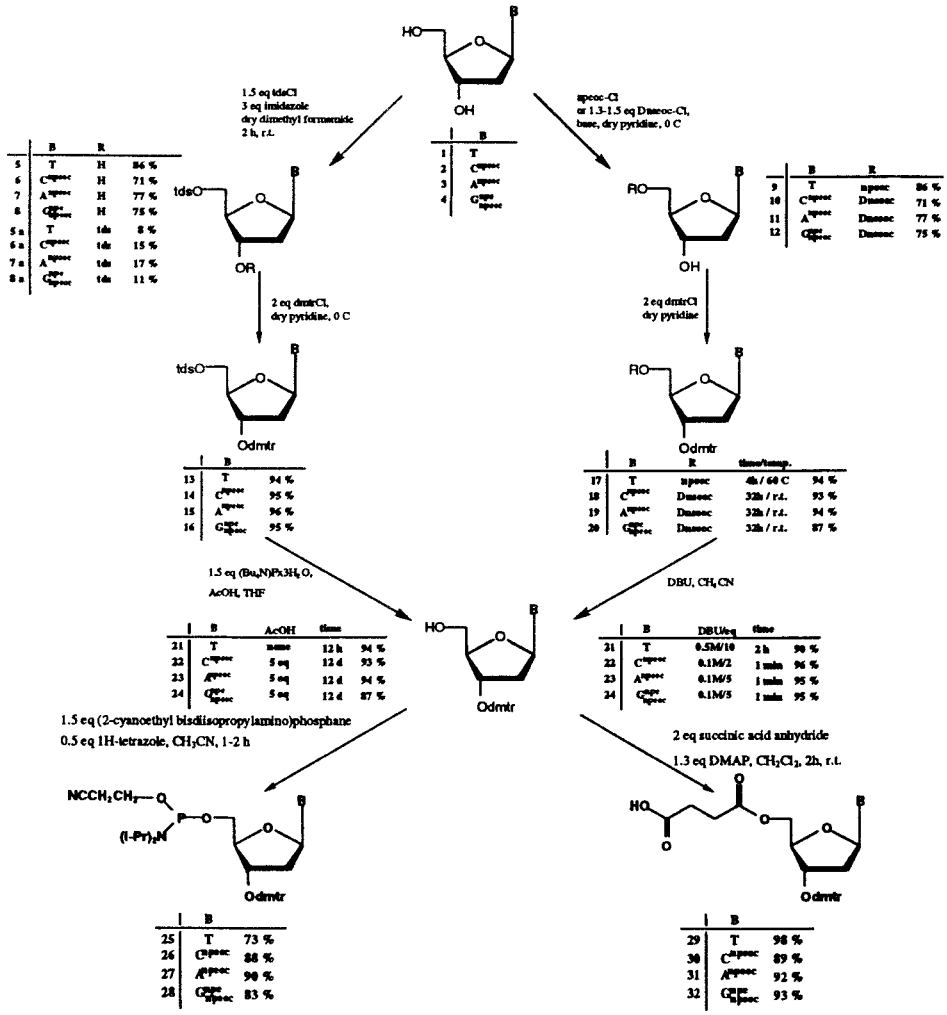
Abstract: We synthesized 3'-O-dimethoxytrityl-5'O-phosphoramidites and 5'-O-succinates which can be used as monomeric building blocks for the built up of oligodeoxyribonucleotides in the alternative 5'-3' direction. With this inverse strategy oligonucleotide 3'-conjugates as well as 3'-3' and 5'-5' internucleotidic linkages can be easily formed.

In nature oligonucleotides are synthesized in the 5'-3' direction, while the chemical built up of these polymeres, due to the easy accessibility of the corresponding monomeric building blocks are carried out in the opposite 3'-5' direction. We now describe the chemical 5'-3' synthesis of oligodeoxyribonucleotides and their related 3'-O-conjugates. For this reason, we would like to report the synthesis of suitably protected monomeric building blocks as well as the assembly of oligonucleotides with a slightly modified cycle.

At first the aglycone functions were protected in the usual manner by the 2-(4-nitrophenyl)ethyl (npe) and the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group[1,2] which have already been proven to be very efficient for the synthesis of oligodeoxynucleotides. The following synthetic modification of the sugar moiety was based on two different four step processes. The 5'-OH function could be blocked by treatment with tdsCl [dimethyl(1,1,2-trimethylpropyl)silyl chloride (= dimethyl(thexyl)silyl chloride)] and imidazole in DMF (-> **5-8**). Consequent dimethoxytritylation at the 3' hydroxyl group led to the fully protected derivatives **13-16**. Due to the basicity of the fluorid ion AcOH had to be added during the desilylation step with $(Bu_4N)Fx3H_2O$ to avoid β-elimination of the aglycone protecting groups. Although an extended reaction time had to be accepted, in this manner the 3'-O-dimethoxytrityl nucleosides **21-24** could be obtained in high yields. In the other route the primary alcohol function was protected with the base labile 2-dansylethoxycarbonyl (=2-{[5-dimethylaminonaphthalen-1-yl]sulfonyl}ethoxycarbonyl; dnseoc}[3-5] and npeoc group to give **9-12**. Again subsequent dimethoxytritylation led to



SCHEME 1: Synthesis of the monomeric building blocks

the 3' and 5' blocked nucleosides **17-20** which afforded by a mild β-elimination process the corresponding 5'-OH derivatives **21-24** in very high yields. Conversion into the corresponding 5'-(2-cyanoethyl N,N-diisopropylphosphoramidites) **25-28** was achieved by reaction with (2-cyanoethyl bisdiisopropylamino)phosphane[6] under *1H*-tetrazole activation. The 5'-O-succinates **29-32** were formed by succinic anhydride and 4-dimethylaminopyridine (DMAP) in almost quantitative yields. The loading of the LCAMA-CPG[7] support was performed with O-{[cyano(ethoxycarbonyl)methylidene]amino}-1,1,3,3-tetramethyl-uroniumtetrafluoro-borate (TOTU) and N-methylmorpholine in MeCN

TABLE 1: Unmodified oligonucleotides and their average condensation yields

Unmodified oligonucleotides	Average condensation yields %
5'-TTG-TGT-GTG-TGT-G-3'	98.6
5'-CCC-CCC-CCC-3'	100.0
5'-AAA-AAA-AAA-3'	99.5
5'-CAC-AGC-GGC-CGC-3'	100.0
5'-TGA-GCA-GA-3'	99.1

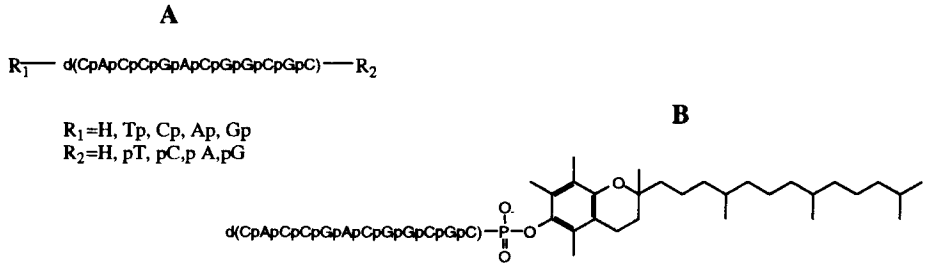


FIG. 1: Modified oligonucleotides

followed by a capping process with Ac_2O and DMAP in pyridine leading to the loaded supports (loading: 29-35 mmol/g).

The oligodeoxyribonucleotides were synthesized using the solid phase phosphoramidite approach by Caruthers and coworkers[8-10] in an Applied Biosystems 392 synthesizer with the common protocoll. In order to achieve high coupling efficiency the coupling time had to be changed to 180 s:

1. Detritylation of terminal dimethoxytrityl group with 3% TCA in CH_2Cl_2 for 135 s

2. coupling with 0.1 M phosphoramidite (25-28) and 0.5 M ^1H-tetrazole in CH_3CN for 180 s

3. capping with Ac_2O/2,6-dimethylpyridine/1-methyl-1H-imidazole in THF for 15 s

4. oxidation with 0.05 M I_2 in THF/pyridine/H_2O for 32 s

(no washing steps are listed)

With this strategy we synthesized unmodified (Table 1) as well as modified oligonucleotides.

The building units of the oligonucleotides modified at the 3' and/or 5' end with an additional nucleotide (FIG. 1; A) were built up in the common 3'-5' manner. By using the 5'-O-succinates and/or the 5'-O-phosphoramidites these 3'-3' and/or 5'-5' internucleotidic linkages between the building units and the additional nucleosides could be obtained. The coupling efficiency of these modification steps ranged between 99.1-99.9%. With this

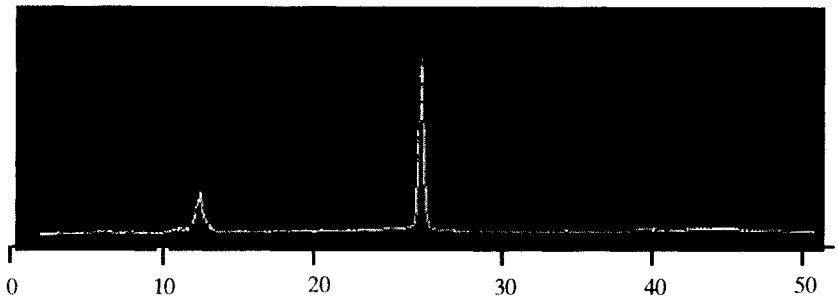


FIG. 2: HPLC of the crude vitamin E 3'-O conjugate

inverse strategy it is also possible to connect monofunctional, lipophilic residues at the 3'-end of oligodeoxynucleotides via phosphoric acid diester linkage. In this manner we synthesized a vitamin E 3'-O-conjugate (FIG. 1; B) and a cholesteryl 3'-O-conjugate. After the built up of the oligodeoxynucleotide at the synthesizer, the attachement of the lipophilic residue to the 12-mer was carried out in an flask under nitrogen with the corresponding phosphoramidites. The finally deprotection step and the cleavage of the 3'-O-conjugate from the solid support was achieved with the usual routine at the ABI 392 synthesizer.
The reverse phase HPLC of the crude vitamin E 3'-O conjugate (FIG. 2) shows the efficiency of the automated solid support synthesis of this inverse approach.

REFERENCES

1 Himmelbach, F.; Schulz, B.S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59
2 Stengele, K.P.; Pfleiderer, W. *Tetrahedron Lett,* **1990**, *31*, 2549
3 Bergmann F,.; Pfleiderer, W. *Helv. Chim. Acta* **1994**, *77*, 203
4 Bergmann F,.; Pfleiderer, W. *Helv. Chim. Acta* **1994**, *77*, 481
5 Bergmann F,.; Pfleiderer, W. *Helv. Chim. Acta* **1994**, *77*, 988
6 Kraszewski, A. ; Norris, K.E. *Nucleic Acids Symp. Ser.* **1987**, *18*, 172
7 Stengele, K.P., Pfleiderer, W. *Nucleic Acids Symp. Ser.* **1989**, *21*, 101
8 Matteucci, M.D., Caruthers, M.H. *Tetrahedron Lett.* **1980**, *21*, 719
9 Beaucage, S.L., Caruthers, M.H. *Tetrahedron Lett.* **1981**, *22*, 1859
10 Matteucci, M.D., Caruthers, M.H. *J. Am. Chem. Soc.* **1981**, *103*, 3185