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PURIFICATION ORIENTATED SYNTHESIS OF OLIGODEOXYNUCLEOTIDES IN SOLUTION

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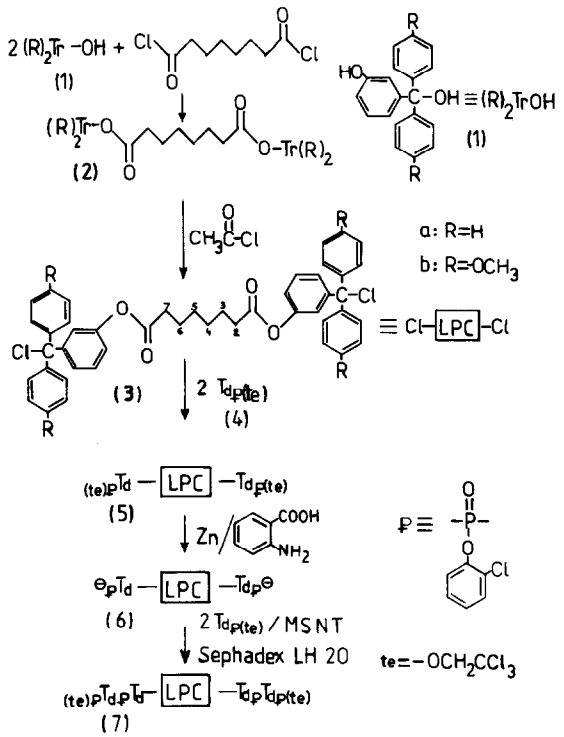
Summary: A liquid phase carrier (LPC) was used as purification handle for the separation of phosphotriester condensation products by Sephadex LH 20 gel filtration chromatography. The carrier consists of an octanedioic acid ester bridge connected to two growing oligonucleotide chains by anchoring on the trityl groups, which increased the molecular size difference between condensation products and the other nucleotidic components of condensation reaction mixtures. By this approach a hexa- and a nonadeoxynucleotide have been synthesized.

At present the method of choice of oligonucleotide synthesis is the application of solid phase carriers[1]. However, for some physico-chemical investigations (e.g. X-ray cristallography and investigations on protein-nucleic acid interactions) larger amounts of oligonucleotide material are needed, which still requires solution synthesis. One of the major drawbacks of current phosphotriester synthesis methodology is the formation of sulfonylated OH-components as side products. Application of one of the most commonly employed condensing agents 1-(mesitylene-2-sulphonyl)-tetrazole (MSTe)[2] or 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole (MSNT)[3] results generally a reaction mixture, from which condensation product, unreacted OH-component and sulphonylated OH-component are sometimes rather difficult or even not separable by standard chromatography techniques on silica gel. Higher resolution in chromatographic purification has been obtained by additional usage of reversed-phase silica gel[4] or by gel filtration on Sephadex LH 60[5].

Sephadex LH 60 and LH 20 are suitable for separations in unpolar solvent systems like alcohol/chloroform. The condensation product is expected to be the first component coming from the column, eluted with the void volume under properly chosen conditions. No losses of product by irreversible adsorption are exptected as is the case in silica gel chromatography. By applying Sephadex LH 20 we expected best purification from sulphonated OH-component (and unreacted OH-component) if large phosphate- and small OH-components are condensed. To increase the difference in molecular size between unreacted phosphate-component and condensation product we introduced into the phosphate component a liquid-phase-carrier (LPC) in such a way, that two oligonucleo-

Scheme 1



tide chains were growing simultaneously on one molecule. The carrier consists of an octanedioic acid bridge which was anchored by ester bonds to the trityl (or dimethoxytrityl) group of the growing chains (scheme 1).

Reaction of 3-hydroxyphenyl-diphenyl-methanol[6] (1a) with octanedioic acid dichloride in pyridine at 0° C for 12 hours yielded the bis-tritanol compound (2a) (70% m.p. 127° C, PMR/CD_3OD/ 1.38/multiplet, 4H, 4,4'-H 5,5'-H/, 1.71/ multiplet, 4H, 3,3'-H 6,6'-H, 2.49/t, 4H, 2,2'-H 7,7'-H/, 6.86-7.26/multiplet, 28 trityl H/; IR:KBr/1740 cm^{-1} -O-C(=O)-, 3550 cm^{-1} -C(-OH)-), which was converted into the bridged bis-tritylchloride (3a) by heating with acetyl chloride under reflux in toluene for 6 hours (90%, m.p. 145° C, el. anal. cal. C 75.92%/H 5.54%/hydrolizeable Cl 9.74%, found C 76.33%/H 5.80%/Cl 9.72%). Tritylation of thymidine-3'-2-chlorophenyl-2,2,2-trichloroethylphosphate (4) with (3a) afforded under rather drastic conditions (100° C, 3.5 hours in pyridine solution) 44% yield of the fully protected LPC-derivative (5a) after short column chromatography. We believe that the decrease in reactivity (3a) compared to unsubstituted tritylchloride is due to the electron-withdrawing effect of the attached ester groups in meta-position. Introduction of two methoxy substituents into each trityl moiety gave the bridged bis-dimethoxytritylchloride (3b), which was obtained as an oil, but could easily tritylate (4) under much milder conditions (with the aid of powdered molecular sieves 4 Å in dichloromethane solution[7]) in 70% yield. The 3'-terminal 2,2,2-tri-

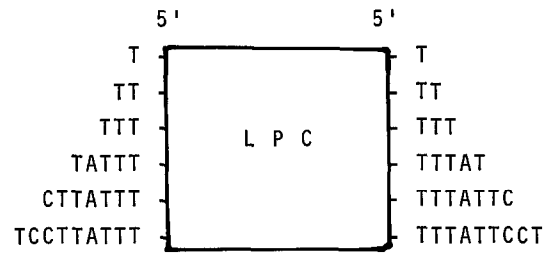


Figure 1: Synthesis strategy leading to fully protected nonadeoxynucleotide by the aid of the liquid-phase-carrier. Benzoyl for adenosine, 2-toluyl for cytidine, 2,2,2-trichloroethyl and 2-chlorophenyl for phosphates and 4-t-butylbenzoyl for 3'-OH were used as protecting groups. Ratio phosphate/ OH-component/MSNT = 1 : 3 : 4,5 - 5.

chloroethyl groups were removed by reductive cleavage with activated zinc powder using the novel coreagent anthranilic acid (10 equiv. Zn/ 5 equiv. anthranilic acid/ 0.05 M pyridine solution[8]) and the resulting LPC-phosphate components were used for chain elongation in 5'-3'-direction leading to fully protected hexamer- and nonamer derivatives (figure 1). MSNT was used as condensing agent. The yields of condensation reaction varied from 85% to 35% with increasing chain length. The condition to achieve optimal yield is being persued. Further experiments are being performed to optimize this method.

Purification of products on Sephadex LH 20 was performed on a 63 x 3.6 cm column (640 cm^3, exclusion volume 200 ml) using chloroform/ethanol (7/3, v/v) as eluent. At a flow rate of 120 ml/hour 2 ml-fractions were collected. Condensation reaction mixture components eluted after having reached the exclusion volume in the following order: condensation products, fractions 1-20, unreacted phosphate-components 15-35, sulphonylated OH-components 30-50, unreacted OH-components 40-75. Only at a higher oligomeric level, where rather long phosphate-components were condensed, a small overlapping in retention time of unreacted phosphate-component and condensation product was observed. This could be overcome by filtration through a very small layer of silica gel.

One elongation cycle included: a) cleavage of 3'-terminal trichloroethyl phosphate protecting group using zinc/anthranilic acid, b) condensation with monomeric or dimeric OH-components, each having 3'-fully protected phosphate moieties (3'-terminal protecting group was 4-tert-butyl-benzoyl), c) gel filtration on Sephadex LH 20 (above trimer level followed by filtration through a layer of 1 cm silica gel). Total time for one elongation step 4 hours.

Complete unblocking was performed by treatment with a) pyridine-2-carboxaldoximate, b) concentrated ammonia, c) 80% acetic acid. The deblocked oligonucleotides were chromatographed on DEAE-cellulose, desalted and sequenced by the mobility shift method (figure 2).

In conclusion the advantages of this purification orientated synthesis

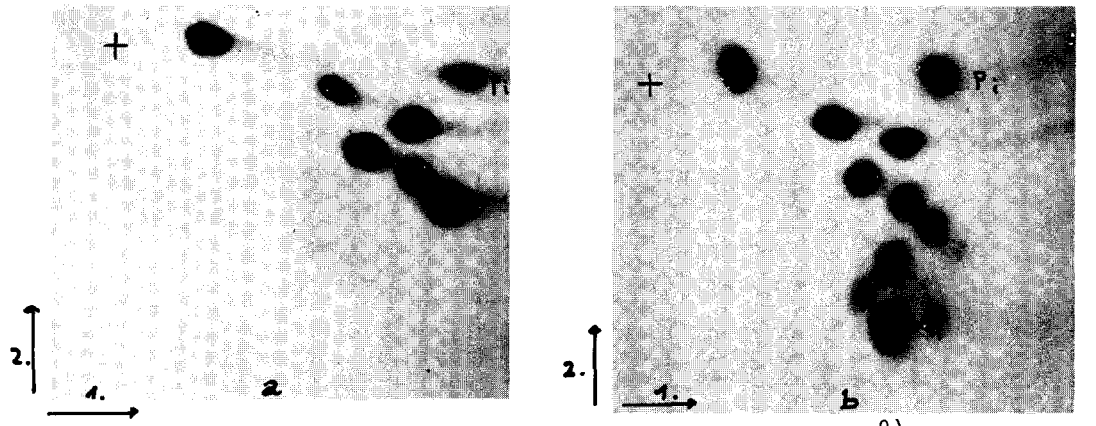


Figure 2: Sequence analysis according to mobility shift method[9].
+: Blue marker, a) d(TTTATT), b) d(TTTATTCCT).

approach are: a) unreacted and sulphonylated OH-components can easily be separated from condensation products, b) the general advantage of the solution-synthesis approach, that high excess (compared with solid phase approach) of one reaction component is not necessary to achieve high condensation yield, c) large scale synthesis is only limited by the capacity of the Sephadex LH 20 column, d) total time of one elongation cycle takes only few hours (depending on the size of the Sephadex column).

Acknowledgment

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