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POLYMER SUPPORT OLIGONUCLEOTIDE SYNTHESIS 13[1]: RAPID AND EFFICIENT SYNTHESIS OF OLIGODEOXYNUCLEOTIDES ON POROUS GLASS SUPPORT USING TRIESTER APPROACH

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ABSTRACT

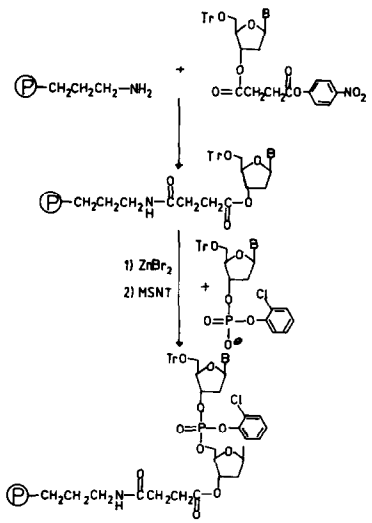
The rapid and efficient synthesis of d(TTTATT) and d(CATGAGGAAGT) on glass beads of controlled pore size using N-acylated 5'-O-trityldeoxynucleoside-3'-(2-chloro)-phenyl phosphates as phosphate components, capping with acetic anhydride, detritylation with $ZnBr_2$ and 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazol as condensing agent is described. The efficiency of monomer and dimer additions is compared.

Since the first synthesis of a longer oligodeoxynucleotide with defined sequence on a polymer support[2] tremendous progress in oligonucleotide chemistry has been achieved[3,4]. Various polymers have been introduced as carriers[5,6]. We now wish to discuss the use of glass beads of controlled pore size (CPG), a carrier which has been first suggested by us when introducing silica gel into polymer support oligonucleotide synthesis[7]. A somewhat similar carrier has been described recently[8].

The advantages of CPG[16] as carrier are:1) In contrast to low cross-linked polystyrene and derivatives of polyacrylamide the accessibility of functional groups on this carrier is not dependent on carefull solvent selection. 2) The carrier has a rigid network, is incompressible and allows the application of high flow rates of solvents and reagents. 3) The pore size distribution is very narrow and the mean pore size can be varied over a wide range (75-3000 Å). 4) After functionalization the surface of the carrier is expected not to interact with the oligonucleotide chains. 5) The carrier is stable under all conditions described and can be reused.

Functionalization and chainextension strategy using phosphate triester approach is shown in scheme 1. Loading of CPG with nucleosides is dependent on pore size. In case of CPG with 1400 Å loading of deoxythymidin was 9.4 μmoles/g, with 240 Å loading is 99 μmoles for deoxythymidin, 97 μmoles N^6-benzoyldeoxyadenosin, 107 μmoles for N^4-(2-methyl)-benzoyldeoxycytidin, and 82 μmoles for N^2-isobutyryldeoxyguanosin /g. As 5'-O-protecting group we used the unsubstituted trityl group which due to its stability makes preparation of intermediates much safer. Its quick removal is possible without any side reaction using the Lewis acid $ZnBr_2$ in $CHCl_3$/MeOH[9] or even better

Scheme 1



diisobutylaluminiumchloride in toluene or hexane which allows removal of trityl groups under completely aprotic and nonpolar conditions[10]. The 2-chlorophenyl group has been used for the protection of phosphate moieties throughout this work. Condensations are mediated by using 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazol (MSNT)[11]. One reaction cycle contains condensation with MSNT, capping with acetic anhydride[12] and detritylation with either zinc bromide or dialkylaluminiumchloride. These reactions are interrupted by appropriate washing steps. Condensation time is 60 minutes for monomer and 90 minutes for dimer addition. Total cycle time is about 2 hours All operations are performed manually in a column with a sintered glass frit and appropriate solvent deliveries using argon pressure. After the last condensation step the 5'-O-trityl group is removed and the polymer is treated with tetramethylguanidinium pyridine-2-aldoximate[11] followed by aqueous concentrated ammonia at 50° C. The total amount of nucleotide material was subjected to a preparative purification using DEAE-cellulose anion exchange chromatography in presence of 7 M urea[13]. Distinct fractions were phosphorylated with T4-kinase and characterized by polyacrylamide gel electrophoresis (20% using a homo-oligo-dT chain length standard[14] and by sequence analysis following the mobility shift method[15]. Results are shown in figure 1 for the hexamer and in figure 2 for the undecamer.

In the case of the hexamer 12-fold excess of phosphate component (0.12 M) has been used. Small amounts of trunket sequences are formed as indicated in figure 1. Therefore the concentration of phosphate components has been enhanced to 0.24 M for the synthesis of the undecamer resulting in almost no trunket sequences (figure 2). Under these conditions constantly high coupling yields were obtained although two dimer additions were included, amongst them the most unfavourable coupling of AG block to G. When using 0.2 g carrier

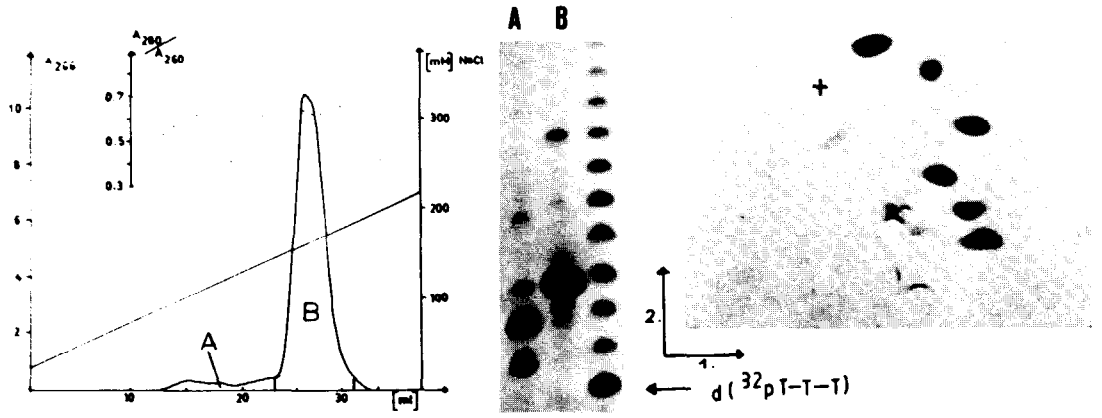


Figure 1: a) DEAE-cellulose chromatography, b) polyacrylamide gel electrophoresis of fractions of DEAE-cellulose chromatography, c) sequence determination of fraction B.

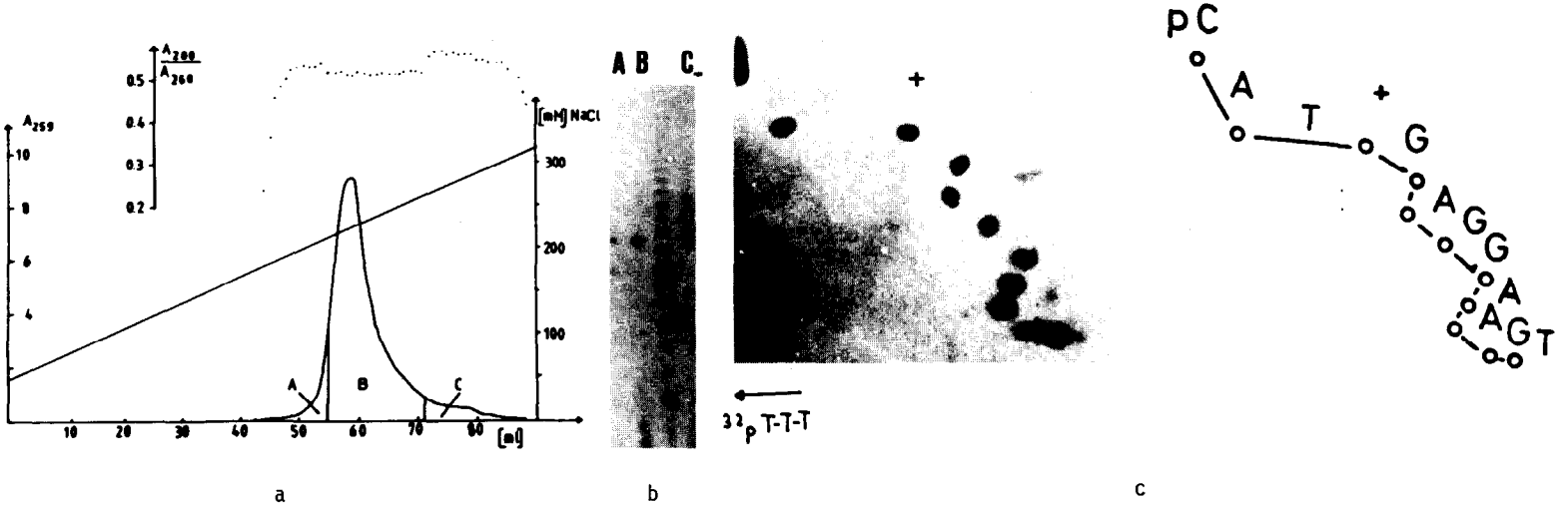


Figure 2: a) DEAE-cellulose chromatography, b) polyacrylamide gel electrophoresis of fractions of DEAE-cellulose chromatography, c) sequence determination of fraction B, X = Blue marker.

115 O.D. pure hexamer and 152 O.D. pure undecamer were obtained. This is 9.5% and 6.3% overall yield of pure material, respectively.

The results demonstrate that controlled pore glass is a highly suitable carrier for oligodeoxynucleotide synthesis. The time for one coupling cycle can be further reduced as a capping step seems to be unnecessary. It is believed that the excess of phosphate component can be reduced, although there is no evident need for it: we found that after extraction with $CHCl_3$/ saturated bicarbonate and precipitation with petroleumether, the phosphate components could be reused for condensation, with coupling rates identical to the unused phosphate components. Further improvements are possible by elongating the spacer length (2 glycine residues have been introduced between the aminopropyl- and succinyl group; A. Stumpe, to be published) and by use of a microprocessor-controlled synthesizer (J.-P. Freundt, M. Fischer, H.-H. Muth, to be published). A significant reduction in cycle time (30 - 40 minutes) has been obtained when using the phosphite triester approach (N.D. Sinha, to be published).

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Literature

+ New adress: BIOSYNTECH Biochemische Synthesetechnik GmbH & Co, Stresemannstraße 268-280, D-2000 Hamburg 50, FRG.
1) Part 12 is: W. Heidmann and H. Köster, Makromol. Chem. 181, 2507 (1980).
2) H. Köster, A. Pollak and F. Cramer, Liebigs Ann. Chem. 1974, 959.
3) H. Köster, Nachr. Chem. Tech. Lab. 27, 694 (1979).
4) M. Ikehara, E. Ohtsuka and A.L. Markham, in: Carbohydrate Chemistry and Biochemistry, Academic Press 1979, 135.
5) H. Köster, Nachr. Chem. Tech. Lab. 29, 230 (1981).
6) N.K. Mathur, C.K. Narang and R.E. Williams, Polymers as aids in organic chemistry, Academic Press 1980, 81.
7) H. Köster, Tetrahedron Lett. 16, 1527 (1972).
8) G.R. Gough, M.J. Brunden and P.T. Gilham, Tetrahedron Lett. 22, 4177 (1981).
9) V. Kohli, H. Blöcker and H. Köster, Tetrahedron Lett. 21, 2683 (1980).
10) H. Köster and N.D. Sinha, Tetrahedron Lett. 23, 2641 (1982).
11) C.B. Reese, R.C. Titmas and L. Yau, Tetrahedron Lett. 19, 2727 (1978).
12) W. Heidmann and H. Köster, Angew. Chem. Int. Ed. 15, 547 (1976).
13) H. Köster, H. Blöcker, R. Frank, S. Geussenhainer and W. Kaiser, Liebigs Ann. Che. 1978, 839.
14) R. Frank and H. Köster, Nucleic Acids Res. 6, 2069 (1979).
15) H. Blöcker and H. Köster, Liebigs Ann. Chem. 1978, 982.
16) Commercially available from Electro-Nucleonics Inc., Fairfield, N.Y., U.S.A.

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