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OLIGONUCLEOTIDE SYNTHESIS ON A POLYMER SUPPORT SOLUBLE IN WATER AND PYRIDINE [1]

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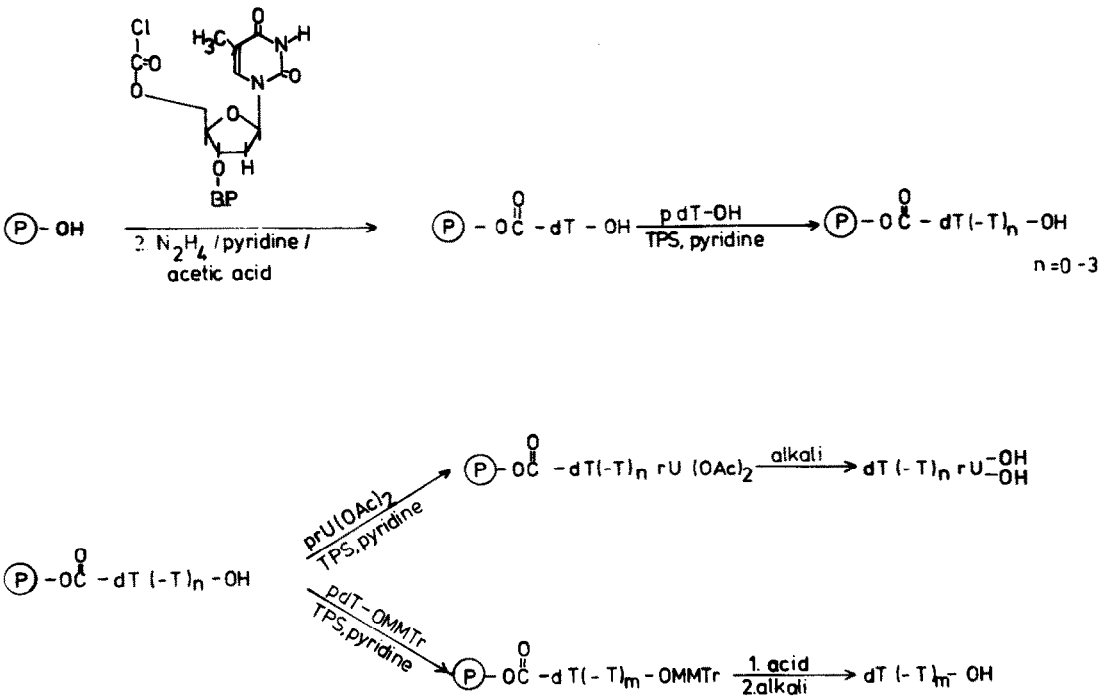
Introduction: Early studies on oligonucleotide support synthesis have centered on the use of polystyrene based carriers soluble in organic solvents [2,3]. These permitted the elongation of the oligonucleotide graft chain in solvents for which a maximum rate of this reaction had been shown, such as pyridine, whereas all reactants and by-products not bound to the support could easily be removed after the condensation by precipitation of the polymer in water. Thus, by repetition of the condensation and precipitation steps oligonucleotides of short chain length were built up in yields which, in some cases, equalled those obtainable in carrier-free condensations. The disadvantage of these systems, however, was that some crosslinking and a change of solubility would take place after going through several chain elongation cycles [4]. Recent advances in the development of techniques for the separation of low molecular weight compounds from water soluble polymers, e.g. dialysis, ultrafiltration, Sephadex chromatography, have led us to investigate on the use of a support system soluble in pyridine as well as in water, thus retaining the advantages and circumventing the disadvantages of the carrier systems described above. Such a support system was developed on the basis of a copolymer of vinylacetate and N-vinylpyrrolidone. This communication describes the preparation of this support system together with some preliminary oligonucleotide studies aiming at the development of a rapid synthesis of short chain building blocks for fragment condensations of polynucleotides and primers for nucleotide polymerizing enzymes. Recently other soluble polymer systems of potential use for nucleic acid chemistry have been reported [5], however, they have not been shown to exhibit the solubility properties inherent to our system in the synthesis of oligonucleotides.

Results and discussion: The polymer support (Ⓟ -OH, scheme 1) was prepared by bulk copolymerization of N-vinylpyrrolidone and vinylacetate with azobisisobutyronitrile as initiator for 10 h at 70° [6] and subsequent saponification of the ester groups in H_2SO_4/t butanol [7]. The molar ratios of monomers were chosen so as to give a product containing between 10 and 20 % vinylalcohol

units. The polymer was purified by dialysis in water and lyophilized. Precipitation from chloroform solution into ether gave a number of fractions from which the one of average molecular weight about 42 000 (determined by viscosimetry) and an alcohol content of 1 mmol/g was chosen for further studies. For attachment of nucleosides 1 g of copolymer, dissolved in 5 ml dimethylformamide and 1 ml triethylamine, was reacted with 2 mmoles 3'-O-ß-benzoylpropionyl deoxythymidine-5'-chloroformate [8] for 24 h at room temperature. The unreacted alcohol groups were then blocked with acetic anhydride in pyridine, each reaction step being followed by dialysis. 960 mg of support were obtained, the nucleoside content of which was determined from UV measurements ($\lambda_{max}^{H_2O}$ = 248 nm, shoulder at 272 nm, ϵ_{248} = 16 400 l/mol · cm) to be approximately 0,11 mmoles/g. After nucleoside deblocking by treatment with hydrazine hydrate [9] the carrier system (Ⓟ-OCO-dT-OH, scheme 1) was ready for oligonucleotide synthesis.

In a test run for nucleotide polycondensations 800 mg Ⓟ-OCO-dT-OH were reacted with 3 mmoles 5'-deoxythymidylic acid in 5 ml pyridine, using 6 mmoles triisopropylbenzene sulfonylchloride as condensing agent. After 72 h at room temperature the mixture was dialyzed in water. For complete removal of the non-bound oligonucleotides chromatography on Sephadex G 100 was necessary. The resulting polymer (Ⓟ-OCO-dT(-T)$_n$-OH) was analyzed for its oligothymidylate content by cleavage of a sample of the support in concentrated ammonia at room temperature and chromatographic separation. The products obtained and their yields are given in table 1. It is clear from these results that the grafting of the oligonucleotide chains to a support favors the formation of short chain lengths, This parallels earlier findings reported by T. Kusama and H. Hayatsu for studies with a derivatized Merrifield resin [4]. With the aim of synthesizing short sequences in a most rational fashion the conversion of the nuncleotidic material on the support was completed by addition of a suitably blocked nucleotide. In one case 320 mg Ⓟ-OCO-dT(-T)$_n$-OH was reacted with 1 mmol 3'-O-p-methoxytrityl deoxythymidine-5'-phosphate (prepared analogously to a method of R.L. Letsinger et al. [10]) and 2 mmoles triisopropylbenzenesulfonylchloride in pyridine for 24 h at room temperature. After dialysis and Sephadex G 100 chromatography the elongated support (Ⓟ-OCO-dT(-T)$_m$-O MMTr, scheme 1) was analyzed for its oligonucleotide content by treating it first with 80 % acetic acid, then with concentrated ammonia and separation of the oligomer chains by chromatography. The yields obtained after this reaction cycle are given in line 2 of table 1. In a similar reaction Ⓟ-OCO-dT(-T)$_n$-OH was elongated by condensation with $O^{2'}$, $O^{3'}$-diacetyl-5'-ribouridylic acid to give, after cleavage, products of the type dT(-T)$_n$-rU (n = 0 - 2). It can be seen from table 1 that a drastic improvement of oligonucleotide yields has been



abbreviations:
(P) = vinylalcohol / N-vinylpyrrolidone copolymer
B.P = ß-benzoylpropionyl-
Ac = acetyl-
MMTr = p-methoxytrityl-
TPS = triisopropylbenzene - sulfonylchloride

scheme 1

Table 1: Yields of products obtained from oligonucleotide syntheses on a support based on a copolymer of N-vinylpyrrolidone and vinylacetate

Products +:	dT	d T-T	d $T(-T)_2$	d $T(-T)_3$
Yield I ++ (Mol %):	70	24	6	1
Yield II ++ (Mol %):	28,5	56	13	2

+ Higher oligomers, present in very small amount (< 1%), were not characterized.

++ Yield I = after polycondensation

Yield II = after stepwise elongation (for conditions see text)

attained which by far exceeds the yield improvement obtained by multiple polycondensations in the work cited above [4]. Thus, although the individual reaction steps have to be further worked out, the fact that a satisfactory conversion of the initially attached nucleoside (about 80 %) was shown in these orienting experiments and that affinity chromatography of the material released from the carrier will easiliy separate the chain extension products bearing trityl [11] or uridine [12] termini demonstrates the applicability of this support system for rapid syntheses of linear oligonucleotides of the general composition A B_n C, where A, B and C can be different deoxy- or ribonucleotides. Studies on the preparation of such oligomeric sequences and their use for the chemical and enzymic synthesis of longer oligonucleotide chains are in progress.

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