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POLYMER SUPPORT OLIGONUCLEOTIDE SYNTHESIS VII [1]

USE OF SEPHADEX LH 20

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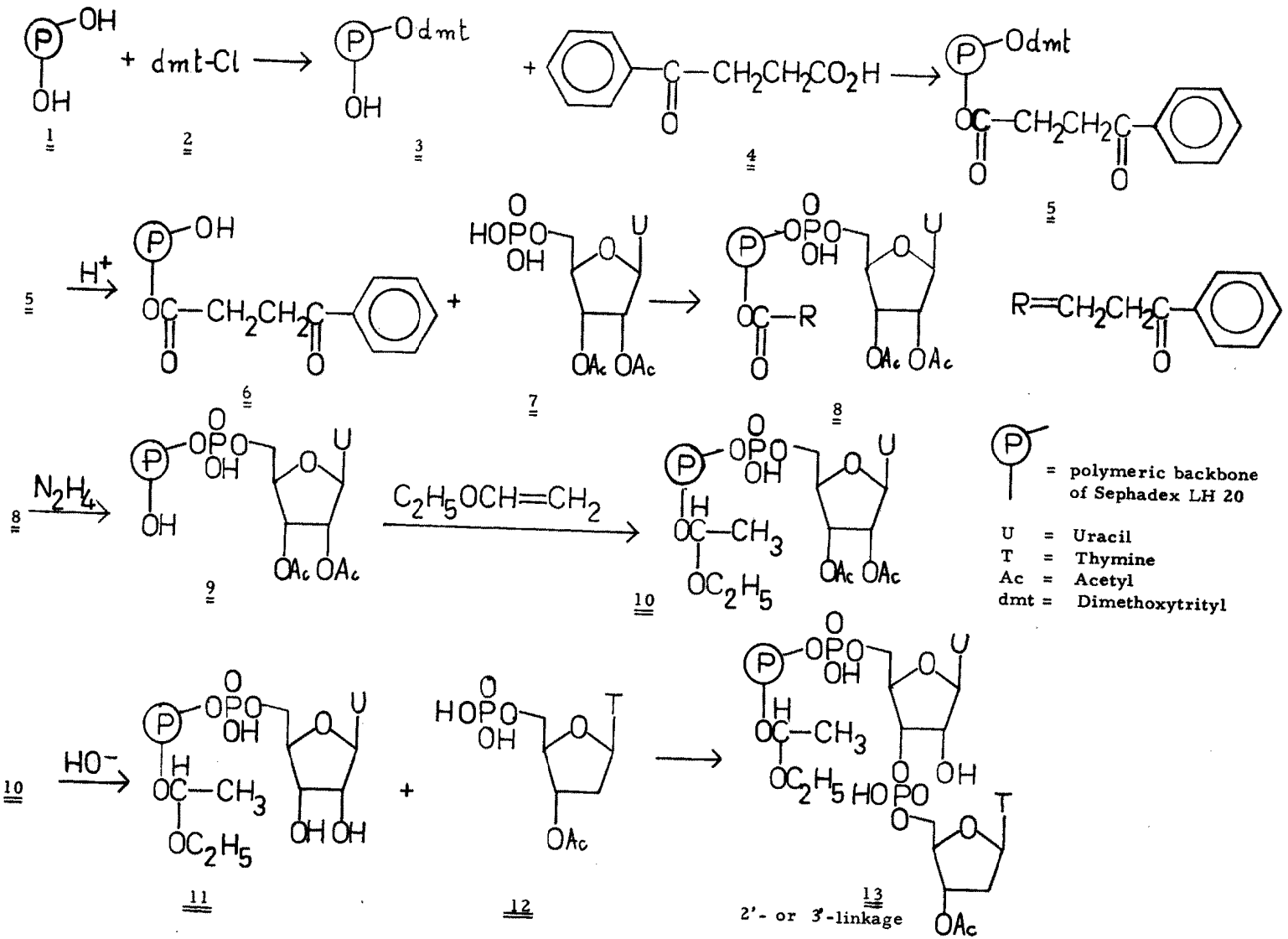
In the preceding paper [1] we described the use of inorganic carriers for the synthesis of oligonucleotides, in our attempt to find suitable hydrophylic carriers, in order to avoid difficulties associated with the high polarity of the phosphodiester bond. In this and the following paper [2] we describe our preliminary results with two organic but very hydrophylic carriers : compounds of the Sephadex series (Pharmacia, Uppsala) and of polyethylenglycol of different molecular weight [2].

We first selected Sephadex LH 20 because of its both hydrophylic and lipophylic character, which should be of advantage, as in the condensation step anhydrous conditions are necessary, while for the removal of blocking groups and for hydrolyzing the synthesized oligonucleotide chain from the carrier, aqueous conditions have to be employed.

Uridine serves as an anchor for the synthesis of deoxyoligonucleotides. We made use of the neighbouring group participation of one of the cis-diol OH-groups in making the phosphodiester bond sensitive to alkali. Thus during deoxyoligonucleotide synthesis one of the two has to be protected by an alkali-stable but acid-labile protecting group (e.g. α-ethoxyethylidene [3]). Having finished the synthesis of a deoxyoligonucleotide chain first the acid-labile protecting group is cleaved off from the 2' - or 3' -position on the uridine molecule bound to the polymeric carrier and second the alkali-stable deoxyoligonucleotide chain is released from the carrier by treatment with alkali. In order to permit the synthesis to proceed only in the desired way all OH-groups of Sephadex L 20 which are not involved in carrying the anchor molecule have to be protected by an alkali-stable blocking group. This should alter the solvolytic properties of the carrier only to a small extent. For this purpose we used the α-ethoxyethyl ether group. Most of the reactions carried out could be followed qualitatively by infrared spectroscopy.

In order to select the sterically less hindered OH-groups we first treated Sephadex LH 20 ($\underline{1}$) with the bulky p.p'-dimethoxytrityl chloride ($\underline{2}$) (10 g $\underline{1}$, 25 g $\underline{2}$ in anhydrous pyridine, 18 hrs., 22°) (IR: 1602, 785, 750, 712, 700 cm^{-1}). $\underline{3}$ was reacted twice with ß-benzoylpropionic acid ($\underline{4}$) [4] and triisopropylbenzene sulfonylchloride (TIPS) in anhydrous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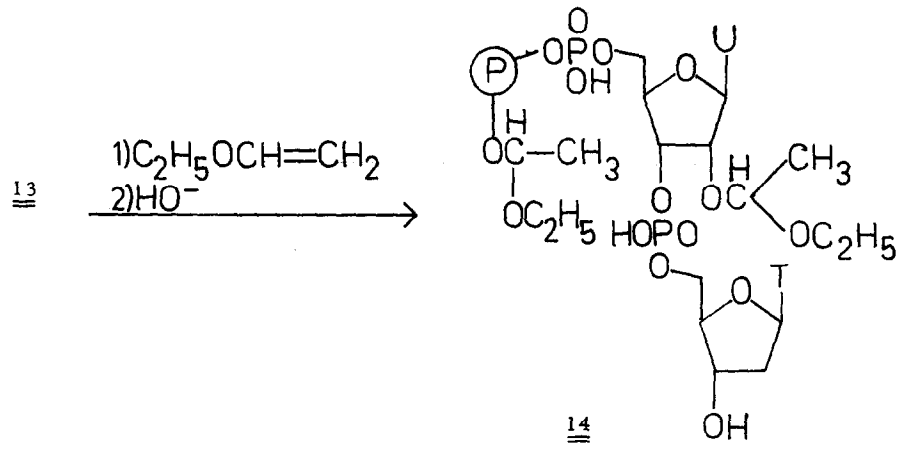
$C_2H_5OCH=CH_2$
= polymeric backbone of Sephadex LH 20
U = Uracil
T = Thymine
Ac = Acetyl
dmt = Dimethoxytrityl
2'- or 3'-linkage

pyridine (22 hrs., 22°) to form 5 (IR : 1682 and 1730 cm^{-1}), which was subjected to acid hydrolysis with 80% acetic acid (6 hrs., 22°) to give 6 (IR: 1602 with less than half the intensity of 3 and 5 , 785 and 712 cm^{-1} diminishes)Now the anchor molecule could be bound to the carrier in a condensation reaction with 2', 3'-O-acetyl-uridine-5'-phosphate (7) and TIPS (anhydrous pyridine, 24 hrs., 22°). In a parallel experiment using 2', 3'-O-[^{3}H]-acetyl-uridine-5'-phosphate (7) it could be calculated from the radioactivity found after alkaline treatment that 95 µmol 7 are bound to the carrier 8 (from N-value of elementary analysis the same value can be calculated). In the IR a broad peak appeared around 1700 cm^{-1} The ß-benzoylpropionyl group was cleaved off with hydrazine/acetic acid/pyridine buffer [4] (20 hrs., 22°) and the regenerated OH-groups of 9 protected with ethylvinylether (0.6% trifluoracetic acid, N, N-dimethylformamide/dimethylsulfoxide = 2:1, v/v, 0°, 24 hrs.)[3] to give 10. The O-acetyl-groups of the uridine moiety could be released (pyridine/3 N sodium hydroxide in methanol = 3 : 1, v/v, 0°, 20 minutes) and the first deoxynucleotide could be condensed with the carrier 11 to form 13 using 3'-O-acetylthymidine-5'-phosphate 12 (12 hrs., 22°, using TIPS as condensing agent in anhydrous pyridine). On steric reasoning, only one of the two OH-groups will react under the conditions used [5].



After subsequent reaction with ethylvinyl ether and alkaline treatment 14 can undergo another condensation step, followed only by removal of the 3'-O-acetyl group. In this way the deoxy-oligonucleotide chain can be built up. 0.1 mmol thymidine could be cleaved off the carrier 13 with 0.1 N sodium hydroxide (20 minutes, 22°), but no nucleotidic material could be hydrolyzed with alkali from the carrier 14 (0.1 N sodium hydroxide, 1 hr., 22°). The identity of thymidine could be shown by tlc on silica gel (Merck, chloroform/methanol = 9 : 1, v/v), paper

chromatography (ammonium acetate, 1 M, pH 7.5/ethanol = 3 : 7, v/v, Schleicher & Schüll 2043 bmgl) and by its UV-spectrum.

The stepwise chemical synthesis of deoxyoligonucleotides with this carrier is now under investigation in this laboratory. It may be noted here that this carrier with uridine as an anchor is suitable for the solid phase enzymatic synthesis of deoxynucleotides [6] with terminal transferase [7] and for the solid phase synthesis of ribooligonucleotides with polynucleotide phosphorylase [8] in a polymerisation reaction and in a stepwise synthesis.

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