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POLYMER SUPPORT OLIGONUCLEOTIDE SYNTHESIS VI[1-5]
USE OF INORGANIC CARRIERS

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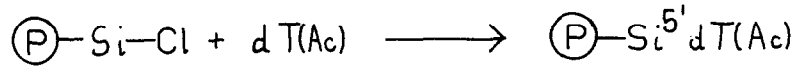
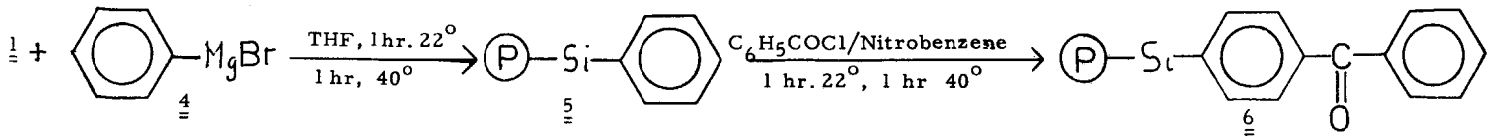
Various methods for the synthesis of oligonucleotides on polymeric carriers are in progress in this laboratory. We first used makroporous, nonswellable polystyrene [1, 3, 5] and were able to synthesize $dT(pdT)_7$ and dTpdTpdApdCpdCpdTpdA [4,6]. In another study we used popcorn polystyrene for the synthesis of $dT(pdT)_5$ [2]. In testing hydrophobic polymeric carriers, which are swellable in organic solvents such as popcorn polystyrene [2], we observed some difficulties when synthesizing an oligonucleotide chain due to the highly polar phosphodiester linkage. We therefore decided to look for hydrophylic polymeric carriers [7] in order to overcome this problem.

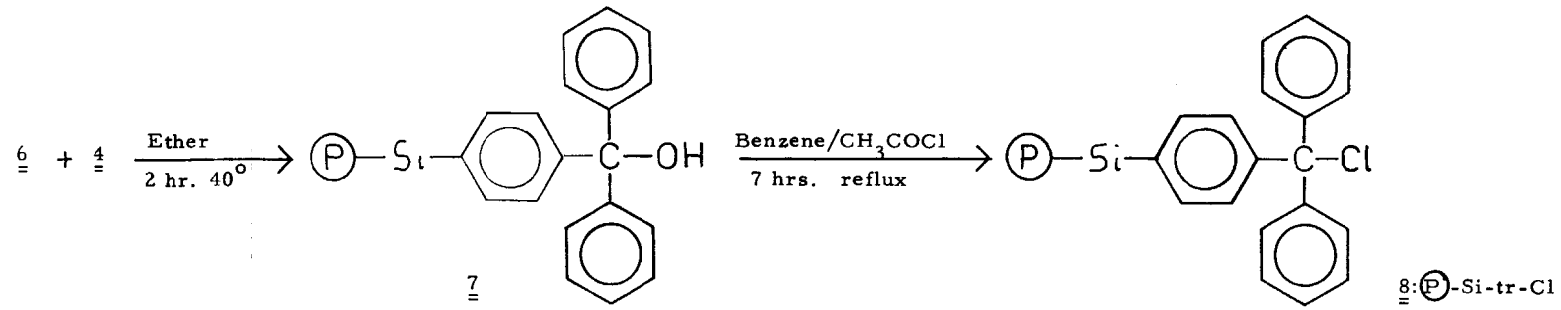
In a first attempt we tried nonporous glass beads (0.02 - 0.05 mm in diameter ; surface $\ll 1\ m^2/g$) which would allow the oligonucleotide chain to be attached only on to the outer surface of the spheres. The glass beads could be condensed with $pU(Ac)_2$ using triisopropylbenzene sulfonylchloride (TIPS) in pyridine(4 hrs. 22°) or could be converted with thionyl chloride/benzene (1 : 2, v/v, reflux for 4 hrs.) [8] into the Si-Cl-derivative $\underline{1}$. This could be loaded with dT(Ac) ($\underline{2}$) in pyridine (20 hrs., 22°). In both cases only 0.1 µmol $pU(Ac)_2$ or dT(Ac) ($\underline{2}$) could be bound per g carrier due to the very small sur-

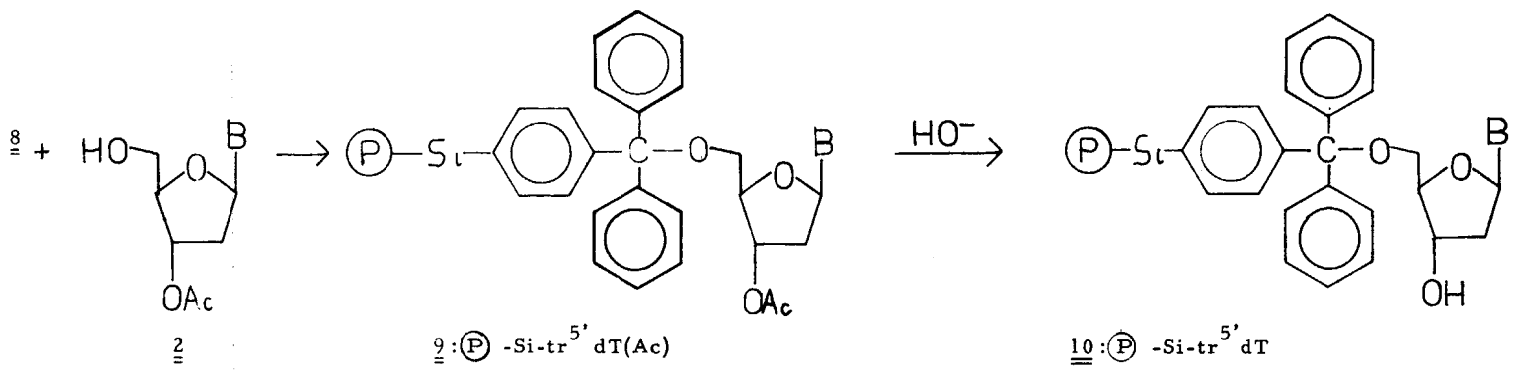


$\underline{1}$ $\underline{2}$ $\underline{3}$

face area. Therefore we used silica gel (Merck, < 0.08 mm in diameter, 427 m^2/g surface area). This could be converted to the Si-Cl-derivative $\underline{1}$ (116 µmol Cl^-/g), which reacted with dT(Ac) ($\underline{2}$) (pyridine, 12 hrs., 22°) in a yield of 5.5 µmol/g. Direct condensation of silica gel and pdT(Ac) using TIPS (1 g silica gel, 0.2 mmol pdT(Ac), 0.5 mmol TIPS, 3 hrs., 22°) gave Ⓟ-Si-pdT(Ac) in a yield of 45.5 µmol/g. In both cases the nucleotidic compounds could be cleaved off either by 0.1 N hydrochloric acid or 0.1 N sodium hydroxide, the reaction being complete within 20 minutes by 22°.







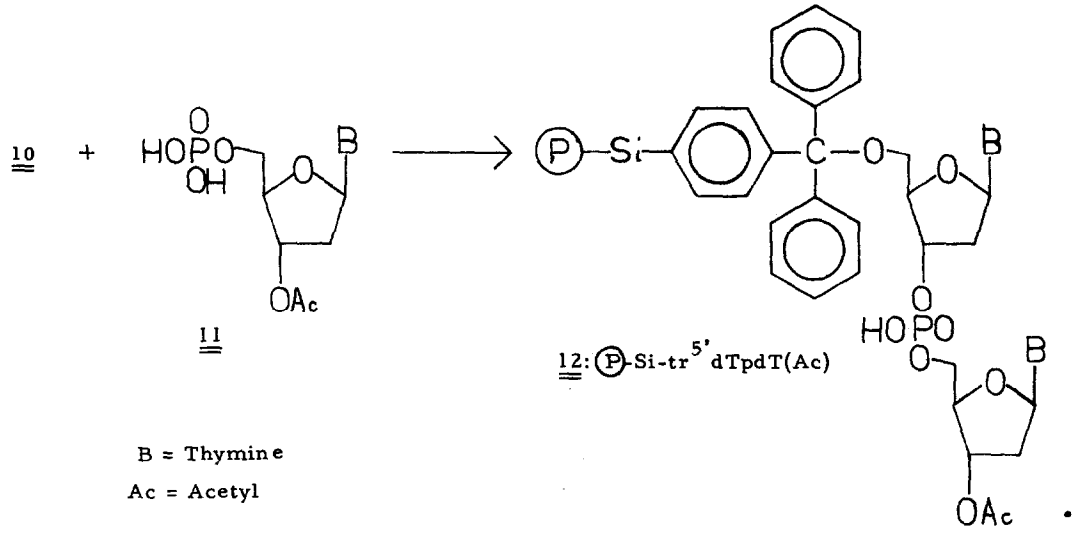
P = polymeric backbone

B = Thymine

Ac = Acetyl

In consequence of the lability of the Si-O-P- and Si-O-C-linkage, which make them unsuitable for oligonucleotide synthesis [9], we converted 1 via the reaction sequence shown into a silica gel carrying a trityl carbinol group (7) which is bound by the more stable Si-C-linkage. By treatment with benzene/acetic acid chloride (reflux, 7 hrs.) 7 can be converted to a polymeric bound trityl chloride group (8) (0.19 mmol Cl^-/g). In a coupling reaction with dT(Ac) (2) 10.7 µmol dT(Ac) could be bound to the carrier 8 to give Ⓟ-Si-tr$^{5'}$dT(Ac) (9).

Cleavage off the carrier 9 can be accomplished using 80% acetic acid (2 hrs., 70°); no more could be cleaved off with 1 N hydrochloric acid (2 hrs., 22°). Under alkaline conditions (0.1 sodium hydroxide, 1 hr., 22°) no nucleotidic material could be cleaved off the carrier 9, however deacetylation was shown by thin layer chromatography (Merck silica gel, chloroform/methanol = 9 : 1, v/v) to be quantitative.



B = Thymine
Ac = Acetyl

Condensation of 10 with pdT(Ac)(11) in pyridine (0.2 g 10, 0.1 mmol 11, 0.25 mmol TIPS, 10 hrs., 20°) gave dTpdT(Ac) in a yield of 54%, along with pdT(Ac) which had reacted with the Si-OH-groups and which was also split off during treatment with 80% acetic acid. This unwanted side reaction can be prevented by protecting the free silanol groups with p-bromomethylphenyl-trichlorsilane [10] before the conversion of 6 to 7. For the synthesis of longer oligonucleotides by this method including the purine nucleotides it might be advantageous to introduce the mono- or dimethoxytrityl chloride group rather than the trityl chloride group in consideration of the enhanced acid lability and to use infusorial earth in consequence of larger pores [11] (surface area 4.2 m^2/g, average pore diameter 11.000 Å, and a small distribution of pore radii) or Bio-glass 2500 (Bio-Rad Laboratories). Work along these lines are in progress.

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4) Part IV in this series
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5) Part V in this series
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6) Abbreviations according to IUPAC-IUB Recommendations,
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T = Thymidine, U = Uridine , d = deoxy , pdT = Deoxythymidine-5'-phosphate,
dTp = Deoxythymidine-3'-phosphate, dT(Ac) = 3'-O-Acetyl-deoxythymidine,
(P)- = Polymeric carrier

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