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SILICA GEL AS SOLID SUPPORT IN THE SYNTHESIS OF OLIGORIBONUCLEOTIDES

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A method is described for the synthesis of ribonucleotides using silica gel as a polymer support. Yields were > 85% at each step in the synthesis of a hexanucleotide containing all four common ribonucleosides.

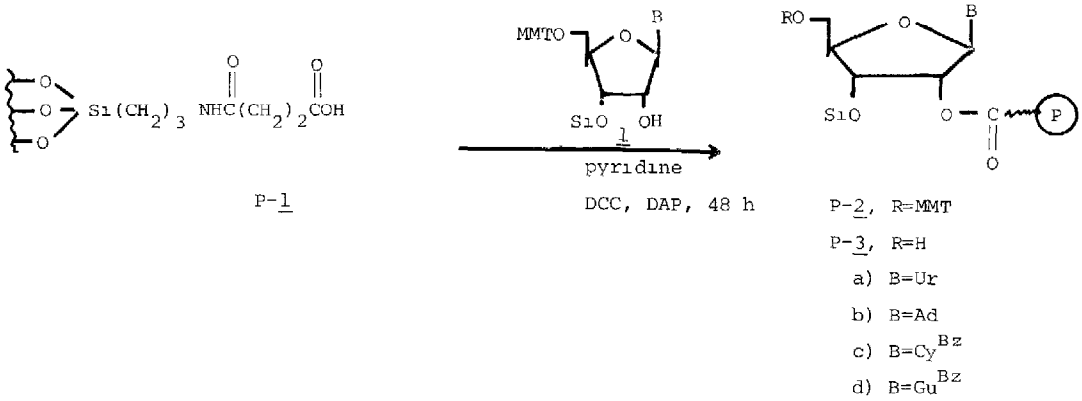
To date the majority of successful syntheses of oligonucleotides have been carried out in solution using variations of the diester method[1] or the triester method[2-4]. However it has been apparent, since the successful introduction of solid phase or "polymer support" synthesis to peptide synthesis[5,6], that this approach could offer numerous advantages in the synthesis of oligonucleotides. Unfortunately a combination of inadequate yields in condensation procedures and the absence of an ideal polymer support has limited the success of the solid phase approach to oligonucleotide synthesis. Several interesting approaches[7-10] in the 1960's clearly outlined the problems to be overcome. Recently a number of laboratories[11-15] have reported very successful advances in the solid phase synthesis of oligodeoxynucleotides. In this report we wish to describe a rapid method for the solid phase synthesis of oligoribonucleotides.

It had long been our opinion that a successful polymer support method would require a rigid non-swellable polymer. This idea was contrary to the majority of polymers being tested in this area. The works of Koster[16] along with the development of surface activated silica gels in general[17,18] convinced us that silica gel could present an ideal support for oligonucleotide synthesis. Our experience[19] with the phosphite condensation procedure[20] further convinced us that this was the ideal procedure for testing, in a solid phase synthesis. Some time ago we began an intensive program to combine these techniques for the development of a fully automated procedure for the synthesis of oligodeoxynucleotides. This goal has been realized and will be described in detail elsewhere. In the meantime Caruthers[14] has described his preliminary results in the deoxy area using a very similar approach.

In this report we wish to describe the synthesis of a ribonucleotide hexamer corresponding to units 21-26 of E.coli $tRNA_f^{Met}$. The hexamer, AGCUCG contains at least one unit corresponding to each of the four common ribonucleosides.

We have tried several silica gels including Merck Kieselgel 60 and Vydak TP silica. The results in this report are based on Vydak TP silica to permit direct comparison with the

reported results in the deoxy area[14]. The polymer is first activated by refluxing HCl and derivatized successively with 3-aminopropyltriethoxysilane, succinnic anhydride and trimethylchlorosilane as has been fully described by others[14,17,18] for the production of polymer 1 (P-1) Treatment of P-1 (6 g) with a 5'-methoxytrityl(-3'-t-butyldimethylsilyl-nucleoside (1, 0.5 mmole/g), DCC (4 6 g) and 4-dimethylaminopyridine (DAP 146 mg) in pyridine (150 ml) with shaking for 48 h produced P-2 The methoxytrityl group was removed with 0 05 N



benzenesulfonic acid (BSA, 20 min) in acetonitrile. The amount of nucleoside covalently bound to the polymer was found to be 0.15 mmole (P-3a), 0 14 mmole (P-3b), 0.17 (P-3c) and 0 16 mmole (P-3d) These amounts were ∿ 5X greater than obtained in the absence of DAP. It was also found that the yields of derivatized polymer were about 30% lower when a 5'-methoxytrityl-2'-t-butyldimethylsilylnucleoside was used, presumably due to greater steric hindrance at the 3'-OH

The general procedure for the synthesis of oligoribonucleotides on the polymer support is as follows To polymer 3d (P-3d, 3 g) was added 0.66 mmole of 5'-0-monomethoxytrityl-2'-t-butyldimethylsilylcytidine which had been previously treated with 0 59 mmole of methyldichlorophosphite in THF (5 ml) containing collidine (0 4 ml) for 15 min at -78°C. The mixture was stirred at -78°C for 30 min and, allowed to warm to room temperature (total 30 min) The phosphite linkage was oxidized to the phosphate by stirring with iodine/water for 10 min at room temperature. The polymer was washed with methylene chloride (15 ml) and ether (10 ml) and dried A sample (100 mg) of the polymer was treated successively with 0.05 N BSA for 20 min and conc. NH_4OH/pyridine (2 1) for 14 h The solution was collected by filtration, concentrated at reduced pressure and the residue was treated with tetrabutylammonium fluoride in THF for 90 min[19] The solution was passed through a Dowex 50WX8 Na^+ column, concentrated and applied to Whatmann 3 mm paper developed in Solvent F (n-propanol-conc NH_4OH-water (55 10 35)) The yield of CpG (90%) was determined spectrophotometrically as % conversion

The remainder of the polymer (2 9 g) was treated with excess phenylisocyanate[11,14] in pyridine for 2 h and was then detritylated with BSA and dried in preparation for the next coupling step. All subsequent coupling steps were carried out as described above and the

% conversions and properties of the oligonucleotides are listed in Table 1 and range from 85 to 98%

Table 1

Compound	R_m^{Tp}	R_f^F	Yield (%)
CpG	0 40	0 39	90
UpCpG	0 44	0 34	98
CpUpCpG	0 59	0 30	85
GpCpUpCpG	0 66	0 25	87
ApGpCpUpCpG	0 76	0 18	86

The tetranucleotide CpUpCpG was degraded with RNAse A to Cp, Up and G in the ratio 2.03 0.96 1 0. The hexanucleotide ApGpCpUpCpG was degraded with RNAse T_2 to Ap, Gp, Cp, Up and G in the correct ratios. Simultaneous treatment with snake venom and phosphatase converted the hexamer to C, U, G and A in the correct ratios.

The purity of the hexanucleotide obtained from paper chromatography in Solvent F was verified by HPLC on μ-Bondapak C_{18} using a linear gradient run from 0% solvent B to 90% solvent B with solvent A. Solvents A and B are those described by McFarland and Borer[21] and give excellent separation of ribonucleotides under the conditions they describe The retention times for the pure pentamer and pure hexamer were 11.1 min and 11.8 min respectively

The results described in this manuscript demonstrate the practicality and efficiency of silica gel as a support for oligoribonucleotide synthesis using the phosphite coupling procedure on silylated ribonucleosides.

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References

1. P.T Gilham and H G Khorana, J Am Chem. Soc , 80, 6212-6222 (1958)
2. R.L Letsinger and K K Ogilvie, J. Am Chem Soc , 89, 4801-4803 (1967)
3. F Eckstein and I Rizk, Angew Chemie, 79, 939 (1967)
4 C B Reese and R. Saffhill, Chem Comm., 767-768 (1968)
5 R.B Merrifield, ibid , 85, 2149-2154 (1963)
6 R L Letsinger and M J Kornet, ibid , 85, 3045-3046 (1963)
7 R L. Letsinger, M J Kornet, V. Mahadevan and D M. Jerina, ibid , 86, 5163-5165 (1964)
8 H. Hayatsu and H G Khorana, ibid , 88, 3880-3887 (1966)
9. L R. Melby and D.R. Strobach, ibid , 89, 450-453 (1967).
10. F Cramer and H Koster, Angew. Chemie, Int. Ed., 7, 473-474 (1968)

11. M.J Gait and R C Sheppard, Nuc Acids Res., 4, 1135-1158 (1977)
12. K Miyoshi and K Itakura, Tetrahedron Letters, 3635-3638 (1979)
13 V K. Potapov, V P Veiko, O N. Koroleva and Z.A Shabarova, Nuc Acids Res., 6, 2041-2056 (1979)
14 M.D Matteucci and M H. Caruthers, Tetrahedron Letters, 21, 719-722 (1980)
15. R Crea and T Horn, Nuc Acids Res., 8, 2331-2348 (1980)
16. H Koster, Tetrahedron Letters, 1527-1530 (1972)
17. J.F. Fritz and J N King, Anal Chem., 48, 570 (1976)
18. P. Tundo and P Venturello, J. Am. Chem Soc , 101, 6606-6613 (1979).
19. K.K Ogilvie, N Theriault and K L. Sadana, ibid , 99, 7741-7743 (1977), K K Ogilvie and N Y Theriault, Can. J Chem , 57, 3140-3144 (1979).
20 R L Letsinger and W B Lunsford, J. Am Chem. Soc., 98, 3655-3661 (1976)
21 G D. McFarland and P N Borer, Nuc. Acids Res., 7, 1067-1080 (1979).

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