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# AUTOMATED OLIGORIBONUCLEOTIDE SYNTHESIS ON SILICA GEL SUPPORTS

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**The oligoribonucleotides $(Ap)_7A$, $(Cp)_7C$, $(Gp)_7G$ and $(Up)_7U$ were prepared via an automated synthesizer using a silica gel support and the methyl dichlorophosphite procedure.**

The rapidly expanding field of molecular biology has created a necessity for synthetic nucleotides of defined sequence. The demand for oligodeoxyribonucleotides has been met by automated syntheses on insoluble polymeric substrates (1-3). However, oligoribonucleotide synthesis has not advanced as far, due to the need for a suitable 2'- hydroxyl protecting group and because of the lower coupling efficiency of ribonucleosides relative to deoxyribonucleosides.

The early oligoribonucleotide syntheses on polymer supports (4,5) were limited to trinucleotide production by the very poor yields of the arylsulphonyl chloride coupling reagents employed. The improved reagents, mesitylenesulphonyl-3-nitrotriazolide and 2,4,6-triisopropylbenzenesulphonyl-3-nitrotriazolide have permitted the synthesis of a heptamer (6) and a tridecamer (7) sequence. However, the limitations of the coupling reagents still required these syntheses to progress by the addition of either dinucleotide or trinucleotide blocks. The need to synthesize these blocks is a severe limit to the ease and speed of a potential synthesis.

Synthesis by stepwise addition of single mononucleoside units is possible by using either nucleoside chloromethylphosphites (8) or nucleoside N,N,-dimethylaminomethoxyphosphines (9). The latter reagent has been used in an automated procedure in conjunction with 2'-O-benzoyl blocked ribonucleosides in THF solution.

We present an automated procedure which uses 2'-O-silyl protected ribonucleosides. Protected adenosine, cytidine, and uridine nucleosides were prepared with the t-butyldimethylsilyl protecting group on the 2'- position (10). Protected guanosine nucleosides were prepared by the reaction of N-benzoyl-5'-monomethoxytritylguanosine with triisopropylsilyl chloride and imidazole in DMF (11). The triisopropylsilyl group allows an easier separation of the resulting 2'- and 3'- silylated guanosine isomers than does the t-butyldimethylsilyl group.

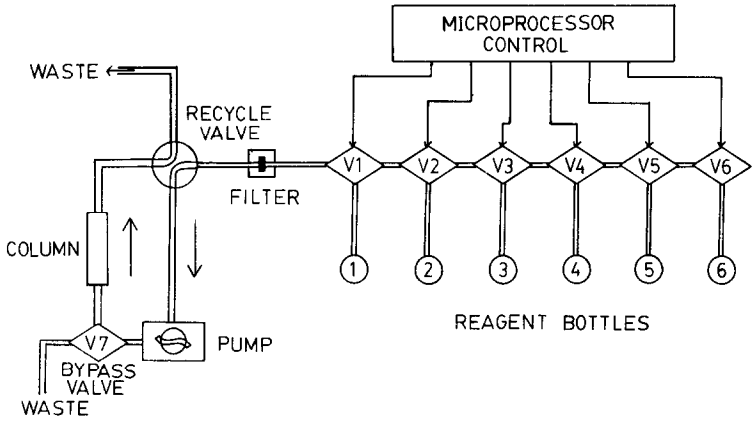


Figure 1: Schematic Diagram of the RNA Synthesizer

Table 1: Synthesis Cycle

| Reagent | Time (@ 4 ml/min) |
|---|---|
| Benzenesulphonic acid (0.1 M) | 5 min |
| Acetonitrile | 1 min |
| Collidine (2%) | 4 min |
| Nucleoside chlorophosphite | 1.25 min |
| Recycle | 23.75 min |
| Iodine (0.1 M), Water:THF:Pyr (2:78:38) | 2 min |
| Acetonitrile | 1 min |
| Acetic anhydride, DMAP, collidine | 5 min |
| Acetonitrile | 5 min |

Table 2: Results of the Octanucleotide Syntheses

| Sequence | Average Coupling Yield | Overall Yield |
|---|---|---|
| TTTTTTTT | 95% | 68% |
| UUUUUUUU | 91% | 52% |
| CCCCCCCC | 92% | 54% |
| AAAAAAAA | 88% | 40% |
| GGGGCGGG | 89% | 45% |

Silica gel (Vydac TP) was derivatized with the 3' terminal nucleoside as previously described (8). The silica gel (175 mg) was packed into a stainless steel column (2.1 mm i.d. x 70 mm L) and subsequent operations were performed by a mechanical synthesizer (Fig. 1) using the coupling cycle shown in Table 1.

The chlorophosphite procedure was used to assemble the oligonucleotides. Nucleosides were phosphorylated by the addition of an acetonitrile solution of the protected nucleoside (1 eq.) to methyl dichlorophosphite (0.9 eq.) and collidine (3.6 eq.) in cooled (-40° to -50°) acetonitrile. The resulting mixture was heated gently to completely dissolve the precipitate and the clear solution was used at room temperature. The nucleoside concentration was approximately 0.03 M and 5 ml of solution was used in each coupling. These solutions were usually prepared fresh each day, although they could be stored in dry ice for several days.

An important feature was the use of acetonitrile as the solvent. Acetonitrile was dried by distillation from $CaH_2$ and gave better coupling results than either pyridine, THF or chloroform.

The machine program was first tested by preparing the octadeoxyribonucleotide $d(Tp)_7T$. The successful completion of this molecule was followed by the synthesis of the four homogeneous oligoribonucleotides, $(Ap)_7A$, $(Cp)_7C$, $(Gp)_7G$ and $(Up)_7U$. Each octanucleotide required only 5.6 hours to synthesize. The yields of each coupling step were determined by measuring the absorbance of the monomethoxytrityl cation released ($E_{470}$= 56,000) by the detritylation step. The average yields of the couplings and the overall yields for each synthesis, as determined by this method, are shown in Table 2.

The octathymidine deoxyribonucleotide was removed from the polymer and deblocked by treatment with 15 M $NH_4OH$/ethanol 3:1 (50°, 16 h). Purification by paper chromatography (12) was sufficient to obtain the final product (Rf 0.06).

The deblocking and purification of the octaribonucleotides was more complicated. The methyl protecting group was removed first (t-butylamine, 50°, 16 h) and then the molecule was cleaved from the silica gel (15 M $NH_4OH$/ethanol 3:1, 50°, 3 h). The adenosine, cytidine and uridine oligomers were purified on silica gel TLC plates (12) at this point. The terminal monomethoxytrityl group easily identified the correct band in each case. Silica gel purification of the guanosine material was omitted because of difficulty in eluting the material from the plates. Recovery of the other nucleotides was about 30%, based on the amount of crude material applied. The silyl groups were removed (1 M TBAF/THF, 3 h), excess TBAF was converted to NaF with $Na^+$ ion exchange resin, and the nucleotide was desalted (Sephadex G-25). The monomethoxytrityl group then was removed (80% acetic acid, 16 h). The completely deprotected material was obtained, by purification on cellulose TLC, as a band with Rf 0.1 - 0.2 (12).

All of the nucleotides were characterized by degradation with Spleen and Snake Venom Phosphodiesterases and, for the pyrimidines, Pancreatic Ribonuclease. The expected nucleotide and terminal nucleoside components were obtained in a 7:1 ratio in all cases with Spleen and Pancreatic Ribonuclease. Degradation with Snake Venom produced only nucleosides because of the presence of a phosphatase impurity. Polyacrylamide gel electrophoresis of $^{32}P$ labelled sequences,

alongside standard nucleotide markers, was also used to confirm the size and the purity of the octanucleotides.

This report describes the results of a systematic study in order to determine the effect, of either (a) different bases, or (b), chain length, on the yields of the chlorophosphite coupling procedure. Pyrimidines gave the best results (average yields of 91 - 92%), while the yields with the purines were slightly less (88 - 89%). Only the yield of the dinucleotide coupling step (approximately 85%) was ever significantly below these averages. This is probably due to a slight inhibition of coupling by the surface of the polymer. For chain extension past the dinucleotide stage, all of the yields were consistently high.

The excellent results of this procedure are currently being extended to the synthesis of mixed sequences of either mRNA or tRNA.

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## References

1, M.D. Matteucci and M.H. Caruthers, J. Am. Chem. Soc. 103, 3185 (1981).
2, G. Alvarado-Urbina, G.M. Sathe, W.C. Liu, M.F. Gillen, P.D. Duck, R. Bender and K.K. Ogilvie, Science 214, 270 (1981).
3, F. Chow, T. Kempe and G. Palm, Nucl. Acids Res. 9, 2807 (1981).
4, K.F. Yip and K.C. Tsou, J. Am. Chem. Soc. 93, 3272 (1971).
5, E. Ohtsuka, S. Morioka and M. Ikehara, J. Am. Chem. Soc. 94, 3229 (1972).
6, E. Ohtsuka, H. Takashima and M. Ikehara, Tetrahedron Lett. 22, 765 (1981).
7, G.A. van der Marel, G. Wille and J.H. van Boom, Recl. Trav. Chim. Pays-Bas 101, 241 (1982).
8, K.K. Ogilvie and M.J. Nemer, Tetrahedron Lett. 21, 4159 (1980).
9, T. Kempe, F. Chow, W.I. Sundquist, T.J. Nardi, B. Paulson and S.M. Peterson Nucl. Acids Res. 10, 6695 (1982).
10, G.H. Hakimelahi, Z.A. Proba and K.K. Ogilvie, Can. J. Chem. 60, 1106 (1982).
11, K.K. Ogilvie, K.L. Sadana, E.A. Thompson, M.A. Quilliam, and J.B. Westmore, Tetrahedron Lett., 2861 (1974)
12, Chromatography on paper or cellulose TLC was performed using nPrOH/$NH_4OH$/$H_2O$ 55:10:35 as the solvent. Chromatography on silica gel TLC was performed using 1 M $NH_4OH$ and ethanol in either 1:9 or 2:8 ratios.

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