Tetrahedron Letters,Vol.23,No.30,pp 3081-3084,1982 0040-4039/82/303081-04$03.00/0
Printed in Great Britain

SEMIAUTOMATED SYNTHESIS OF OLIGONUCLEOTIDES ON A SILICA GEL SUPPORT

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Dodecadeoxynucleotides have been synthesized by the phosphotriester method on a column of γ-aminopropyl silica gel or benzylaminopolystyrene by using a programmed synthesizer.

Polymer support syntheses of oligodeoxyribonucleotides have been improved by several groups using the phosphotriester[1-3] and phosphite[4-6] methods. Polyacrylamide, polystyrene and silica gel are the most commonly used in the phosphotriester or phosphite approach. Silica gel supports are thought to be suitable to place in a column and have proved to be successful in the phosphite method. In this communication we wish to report the phosphotriester synthesis of a dodecadeoxynucleotide by condensation of mononucleotides on a column of silica gel using a programmed machine. A similar reaction was performed on a polystyrene support with a less successful result.

dCATATTCATCGC was synthesized on Porasil C containing γ-aminopropyl group[6] (0.183 mmol/g) first by condensing the 3'-terminal 5'-dimethoxytrityl-N-benzoyldeoxycytidine 3'-succinyl-pentachlorophenyl ester[2] (5 fold excess) in DMF in the presence of triethylamine for 24 hr. The scheme is shown in Chart 1. Unchanged amino groups were blocked by treatment with acetic anhydride, pyridine and dimethylaminopyridine (1:9:0.05). The silica gel (106 mg, 10 μmol dC content, estimated using $\varepsilon_{499} = 7.17 \times 10^4$ in 3:2 $HClO_4$-EtOH) was placed in a column and fitted to a synthesizer (Solid Phase Synthesizer Model 25A, Genetic Design Co., Chart 2). Condensation of 5'-dimethoxytrityl-N-protected nucleoside 3'-(o-chlorophenyl) phosphates was performed as summarized in Table I. The condensation cycle started from the acetic anhydride treatment which removed moisture in the system and blocked unreacted functional groups. The dimethoxytrityl group was removed by treatment with benzenesulfonic acid prior to injection of a mixture of mononucleotides and a condensing reagent (mesitylenesulfonyl 3-nitrotriazolide, MSNT). The yield of each step was estimated by measuring dimethoxytrityl alcohol. It was found that excess of benzenesulfonic acid inhibited the coloring of dimethoxytrityl cations, yields could be underestimated except for the last step. The yield of the last step was estimated by treatment of an aliquot of the support and the resut is summarized in Table II.

Removal of the support from the product was performed by treatment with tetramethylguanidium 2-pyridine aldoxamate[7] in dioxan-water (1:1) for

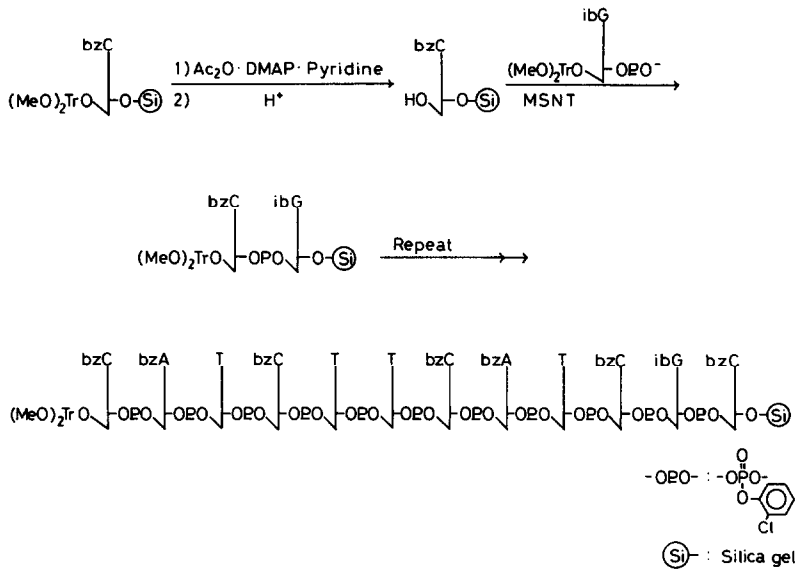

Chart 1

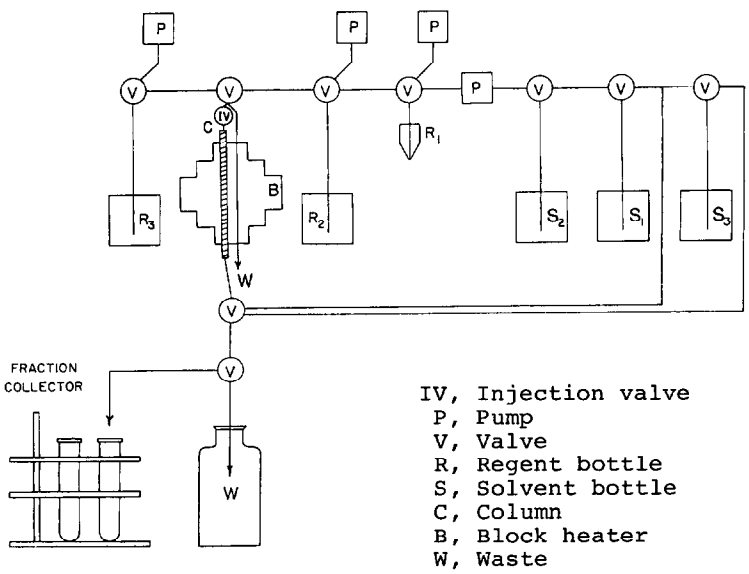

Chart 2

Table I REACTION CYCLE

Step	Solvent or Reagent	Flush, Rest, or Recycle	Collect or Waste	Time (min)
1	Pyridine	flush	waste	5
2	Ac_2O-DMAP-Pyridine(1:0.05:9)	flush	waste	3
3	—	rest	—	5
4	Ac_2O-DMAP-Pyridine(1:0.05:9)	flush	waste	3
5	—	rest		
6	Ac_2O-DMAP-Pyridine(1:0.05:9)	flush	waste	3
7	Pyridine	flush	waste	3
8	CH_2Cl_2	flush	waste	10
9	2% BSA in CH_2Cl_2-MeOH (7:3)	flush	collect	1
10	CH_2Cl_2	flush	collect	1
11	2% BSA in CH_2Cl_2-MeOH (7:3)	flush	collect	1
12	CH_2Cl_2	flush	collect	5
13	CH_2Cl_2	flush	waste	5
14	Pyridine	flush	waste	3
15	N_2	flush	waste	3
16	Nucleotide & MSNT in Pyridine	rest	—	40
17	—	recycle	—	15
18	Pyridine	flush	waste	5

Table II

Chain length	Sequence of linked oligonucleotide	$(MeO)_2$TrOH (A_{499})	Amount (μmol)	Yield (%)	Overall yield (%)
1	HOC-	717	10.0		
2	HOGC-	325	4.53	45.3	45.3
3	HOCGC-	196	2.73	60.3	27.3
4	HOTCGC-	173	2.41	88.3	24.1
5	HOATCGC-	176	2.45	101.7	24.5
6	HOCATCGC-	142	1.97	80.4	19.7
7	HOTCATCGC-	128	1.79	90.9	17.9
8	HOTTCATCGC-	120	1.67	93.8	16.7
9	HOCTTCATCGC-	93.1	1.30	77.8	13.0
10	HOTCTTCATCGC-	86.1	1.20	92.3	12.0
11	HOATCTTCATCGC-	62.1	0.87	72.5	8.7
12	$(MeO)_2$TrOCATCTTCATCGC-	68.2	0.95	109.3	9.5

48 hr. This procedure also removed the o-chlorophenyl groups. The filtered solution was then concentrated and treated with concentrated ammonia to remove N-acyl groups. The 5'-dimethoxytritylated product was separated by reverse phase chromatography (C-18) and deblocked with 80% acetic acid. The deblocked dodecamer dCATCTTCATCGC was further purified by the same chromatography and characterized by mobility shift analysis. Thus the dodecamer was obtained by a simple procedure of mononucleotide condensation in an overall yield of 10% and isolated by a standard procedure of chromatography.

Another dodecamer dCATCTTCATTGC was synthesized by the similar procedure on benzylamino polystyrene [3] using the same machine. The polystylene support was mixed with silanized glass beads (20:1) and packed in a column containing the same glass at the bottom. The overall yield was found to be 6 %. Removal of the support from the product was not completed by the procedure described above and the dimethoxytrityl group was detected on the support after oximate treatment. This may mean that more lipophilic condition is nescessary for the polystyrene support.

Condensation involving di- and trinucleotides on the silica gel support using the synthesizer were performed and the results will be reported elesewhere.

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(Received in Japan 24 April 1982)