Communications to the Editor

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SOLID-PHASE SYNTHESIS OF DEOXYRIBOOLIGONUCLEOTIDES BY THE PHOSPHOTRIESTER METHOD EMPLOYING A NEW POLYMER SUPPORT

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A new polymer-support containing p-nitrobenzophenone oxime groups was prepared from the polystyrene-2% divinylbenzene with p-nitrobenzoyl chloride. The polymer was found to be suitable for deoxyribooligonucleotide synthesis by the phosphotriester method. Synthesized deoxyribooligonucleotide was cleaved readily using hydrazine from the polymer under mild conditions.

KEYWORDS——solid-phase synthesis; polystyrene-bound p-nitrobenzophenone oxime; deoxyribooligonucleotide synthesis; oxime ester; hydrazinolysis; reverse phase column chromatography

Recently, several methods for the polymer-support synthesis of oligonucleotides have been described by a number of laboratories.[1-6] Various polymers such as polydimethylacrylamide,[1] polyacrylmorpholine,[2] polystyrene,[3] silica gel,[4] and porous grass[5] have been applied to the synthesis of oligonucleotides. However, there still remain the problems that the loading amounts of nucleosides on these resins were relatively low and the use of silica gel was undersirable because early coupling yields were much poorer.[7]

In this paper, we wish to report the utilization of a new polymer-support containing p-nitrobenzophenone oxime (3) for solid-phase synthesis of deoxyribooligonucleotides containing N^6,N^6-dibenzoyldeoxyadenosine[8] at the 3'-terminal end by the phosphotriester method.

From commercially available polystyrene (1) (polystyrene-2% divinylbenzene copolymer, 200-400 mesh, Eastman Kodak Co.), a polymer (2) bearing a p-nitrobenzophenone function was prepared by the reaction with p-nitrobenzoyl chloride in the presence of $AlCl_3$. The IR spectrum of 2 had a strong carbonyl absorption at 1665 cm^{-1}, and the elemental analysis indicated that the resin contained 1.20% nitrogen. This shows that one gram of the polymer contained 0.76 mmol of the p-nitrobenzophenone group. The p-nitrobenzophenone polymer 2 was converted to the corresponding oxime 3 according to the Kaiser procedure[9] by treatment with hydroxylamine hydrochloride in a mixture of pyridine and ethanol under reflux. The elemental analysis of 3 showed the presence of 0.76 mmol of the oxime function per one gram of the polymer. This shows that the ketone was converted completely to the oxime.

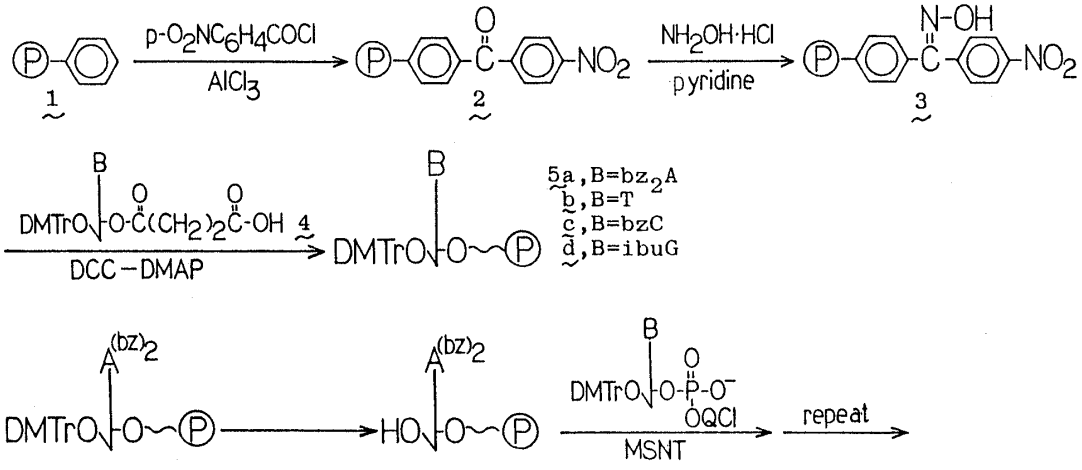
Introduction of 5'-O-dimethoxytrityl-N-protected nucleosides to the oxime resin 3 was performed as follows: 5'-O-Dimethoxytrityl-N-protected nucleosides were converted to their 3'-O-succinate derivatives (4) according to the method of Itakura.[3] The deoxyadenosine 4a (655 mg, 0.79 mmol) was introduced to the oxime resin 3 (500 mg, 0.38 mmol) with DCC (235 mg, 1.14 mmol) in the presence of 4-(di-

methylamino)pyridine (DMAP) (77 mg, 0.79 mmol). After 15 h the resin was washed successively with CH_2Cl_2, DMF, iso-PrOH, MeOH, and pyridine, and the unaltered oxime groups were blocked by reaction with Ac_2O-pyridine-DMAP at room temperature for 2 h. The amount of the deoxyadenosine attached to the oxime resin 3 was determined to be 365 μmol/g by spectrophotometric measurement of dimethoxytritylcarbinol.[10]

In a similar manner, the thymidine (4b), N^4-benzoyldeoxycytidine (4c), and N^2-isobutyryldeoxyguanosine (4d) were introduced to 3, and the amounts of the linked deoxynucleosides were 435 μmol/g, 395 μmol/g, and 406 μmol/g, respectively. The loading amount of deoxynucleosides on 3 is estimated to be about 2 to 3 times higher than those of the usual resin (aminomethyl polystyrene, silica gel, polydimethylacrylamide).

Removal of deoxynucleosides from the oxime resin 5 was examined and it was found that the reaction was successfully carried out by treatment with 0.5 M anhydrous hydrazine in CH_2Cl_2-MeOH (2:1, v/v). The oxime resin 5b was treated with 0.5 M anhydrous hydrazine in CH_2Cl_2-MeOH (2:1, v/v) at room temperature for 15 min. 5'-O-Dimethoxytritylthymidine was obtained by thin layer chromatography in 95% yield. This shows that the nucleoside can be rapidly removed from the oxime resin 5 by treatment with anhydrous hydrazine.

In order to know the effectiveness of the resin 3 as a support, the synthesis of a part of the Dynorphine gene d-GACGAAGGAA[11] was attempted by repeating the reaction cycles summarized in Table I. For the coupling reaction, a mixture of 5'-O-dimethoxytrityl-N-protected deoxynucleoside 3'-(5-chloro-8-quinolyl) phosphates[12] (180 μmol), which were dried by coevaporation with anhydrous pyridine twice, and mesitylenesulfonyl-3-nitro-1H-1,2,4-triazole (MSTN)[13] (540 μmol) in 0.8 ml of anhydrous pyridine was added with a syringe to the oxime resin 5a (50 mg, 18 μmol). The yield of each coupling reaction was estimated by colorimetry of the content of dimethoxytritylcarbinol according the procedure of Ikehara.[7a] The results are listed in Table II. The overall coupling yield for the 10 mer containing a 3'-terminal deoxyadenosine was as good as 45%.



d-DMTrOGACGAAGGAA(bz)2-P → 1) NH_2NH_2 2) $(AcO)_2Zn$ 3) conc.NH_4OH 4) C_{18} column 5) 80% AcOH 6) HPLC-C_{18}

d-GACGAAGGAA

Table I. One Cycle of Operation

Step	Solvent or reagent	Time/min	No. of operation
1	3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5)	1	3
2	CH_2Cl_2	0.5	3
3	Pyridine	1	3
4	Vacuum (oil pump)	10	1
5	Coupling reaction	90	1
6	Pyridine	1	5
7	Ac_2O-DMAP-Pyridine	5	3
8	CH_2Cl_2	1	3

Table II. Synthesis of the 10 Mer Containing a 3'-Terminal Deoxyadenosine

Chain length sequence of linked oligonucleotides	Yield (%)	Overall yield (%)
HOA		
HOAA	95	95
HOGAA	90	85
HOGGAA	90	77
HOAGGAA	87	67
HOAAGGAA	97	65
HOGAAGGAA	91	59
HOCGAAGGAA	88	52
HOACGAAGGAA	87	45
DMTrGACGAAGGAA	100	45

Finally, the complete deprotection of the 10 mer was performed by the following procedure: Oxime resin (10 mg) containing the 10 mer was treated first with 0.5 M anhydrous hydrazine in CH_2Cl_2-MeOH (2:1, v/v, 2 ml) at room temperature for 30 min. The filtered solution was then concentrated and treated with zinc acetate in pyridine-H_2O (9:1, v/v) at room temperature for 2 days to remove the 5-chloro-8-quinolyl groups.[14] The mixture was treated with Dowex 50W-X2 (pyridinium form) and the resin was removed by filtration. The filtrate was concentrated and treated with conc. ammonia-pyridne (5:1, v/v, 6 ml) at 60°C for 6 h to remove the N-acyl groups. The 5'-O-dimethoxytritylated product was separated by reverse phase chromatography [C_{18}, Waters (Fig. 1)] and the DMTr group was deblocked with 80% AcOH (10 ml). The deblocked 10 mer, d-GACGAAGGAA was separated by high pressure liquid chromatography [Radialpak C_{18} silica gel (Fig. 2)] with high purity as proved by electrophoresis [20% polyacrylamide gel (Fig. 3)]. Thus, d-GACGAAGGAA was isolated in 18% yield and the structure of the 10 mer was confirmed by degradation with spleen phosphodiesterase to d-A, d-Cp, d-Gp, and d-Ap in a ratio of 1.0:0.9:4.1:3.9.

In conclusion, it has been found that the resin 3 possesses the following advantages over other support polymers for deoxyribooligonucleotide synthesis; high loading amount of nucleosides on the resin 3, high overall coupling yields, and rapid removablity of the fromed oligomer from the support by use of anhydrous hydrazine.

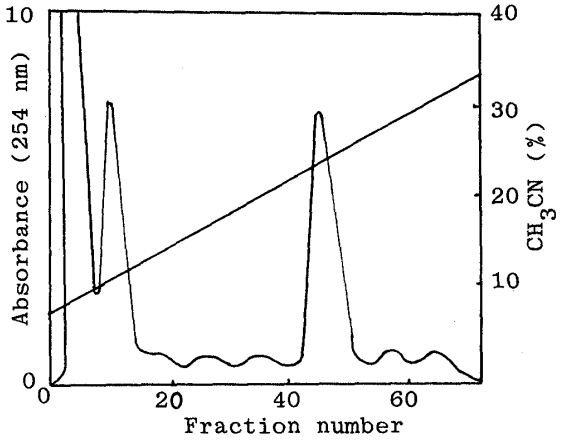


Fig. 1. Chromatography of the 5'-O-Dimethoxytritylated Product on a Column (0.5 X 10 cm) of C_{18} Silica Gel Elution was performed with 10-35% CH_3CN gradient in 0.05 M triethylammonium acetate (total 300 ml).

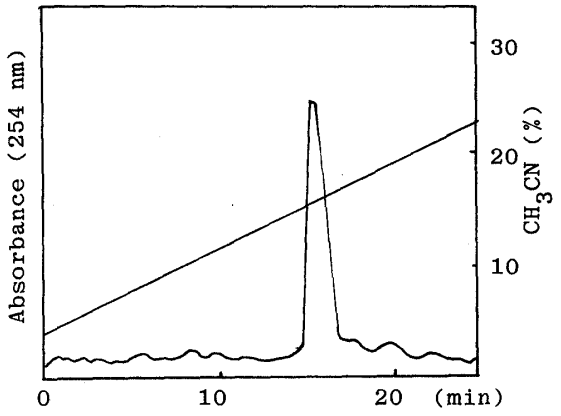


Fig. 2. HPLC Elution Profile on Radialpak C_{18} Coulmn Using a 5-25% Acetonitrile Gradient in 0.01 M Triethylammonium Acetate in 32 min

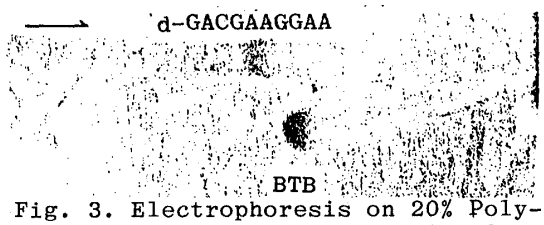


Fig. 3. Electrophoresis on 20% Polyacrylamide Gel under Denaturing Condition (7 M Urea) after HPLC Run

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