SOLID PHASE SYNTHESIS OF $^{5'}$d(AGCAAAAGCAGG)$^{3'}$ USING 9-PHENYLXANTHEN-9-YL AS 5'-PROTECTING GROUP FOLLOWING THE PHOSPHOTRIESTER APPROACH.

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Several solid phase synthesis of oligodeoxynucleotides of defined sequences have been recently described in the literature[1]. We wish to report a solid phase synthesis of the dodecanucleotide long DNA segment of defined sequence, containing over 84% of purine bases, which is complementary to the 3'-end of influenza virus mRNA. In the present methodology, we have used a partially hydrolyzed derivative of cross-linked polyacrylmorpholide, Enzacryl gel K2 of 40-75M mesh size[2].

Immobilization of first 5'-protected dimer block from the 3'-end

To the well stirred suspension of partially hydrolyzed Enzacryl gel K2 (2g) and d[Px-GpGOH][3,4] (I) (50mg, 0.04mmol) in dry pyridine (5ml), 1-mesitylenesulphonyl-3-nitro-1,2,4triazole (MS-NT)[4,5] (0.88g, 3.0mmol) was added and the reaction mixture was stirred for 2 h. The unreacted carboxyl groups of the polymer were blocked by adding dry methanol (0.5 ml) and stirring for 1h at 20°C. The solvent was filtered off and the solid phase was washed with pyridine (2x25ml), CH_2Cl_2 (4x25ml) and 10% MeOH-$CHCl_3$ (25 ml). The solid phase was then suspended in 10% MeOH-$CHCl_3$ containing 3% 4-toluenesulfonic acid. H_2O (8 ml, 0.12 mmol) and was stirred for 60 seconds at 20°C. The solvent was filtered off and the solid phase was then washed with 10% MeOH-$CHCl_3$ mixture (3x10ml) and CH_2Cl_2 (3x10 ml). This depixylation procedure[3] was repeated twice more. The $CHCl_3$ washings from all three depixylation treatments were pooled and extracted with satd. $NaHCO_3$ (Ca. 3x60 ml). The $CHCl_3$ layer was then concentrated and was dissolved in a known volume of $CHCl_3$ to measure the optical density which gave an yield of the first step of condensation to be 0.012 mmol (35%).

Condensation of d[Px-CpAp] (general formula:(2)) to the 5'-OH end of the immobilized dimer on the solid support.

The solid phase from the first step was washed with pyridine (2x10ml) and then it was suspended in dry pyridine (5 ml) along with d[Px-CpAp][3,4] (28.8 mg, 0.02 mmol). To this suspension MS-NT (0.88g, 3.0 mmol) was added and was stirred for 2 h. at 20°C. Then a standard work-up followed by depixylation gave the 5'-OH tetramer which is being hooked on the solid support.

In the same way 5'-protected dideoxynucleoside phosphate-3'-O-phosphodiester triethylammonium salt[3] d[Px-ApGp] (30.7 mg, 0.02 mmol), d[Px-CpAp] (28.8 mg, 0.02 mmol) and d[Px-ApGp] (30.7 mg, 0.02 mmol) were reacted sequentially to elongate the chain length to give rise to the desired deoxyoligonucleotide on the solid support.

Deblocking, purification, isolation and characterization of the dodecamer

The dodecamer was deblocked following a standard procedure[5] and then it was isolated by H.p.l.c. (Permaphase AAX, linear gradient: 0.01 M KH_2PO_4, 0.0M KCl to 0.05M KH_2PO_4, 0.7 M KCl at 60°C, pH 4.5) as shown in fig. 1. Fractions of the last peak were collected and labelled by γ-^{32}P-ATP and kinase. They were then run on a electrophoretogram on 20% polyacrylamide plate. The purity of the fractions are shown in fig. 2; Chanel A: crude mixture before H.p.l.c.; Chanel B: FractionII; Chanel C: Fraction I; and Chanel D: Fraction III. The pure product in fraction III was shown to be the right material of -11 charge by co-running with the oligothymidylic acid markers[6] on gel electrophoresis.

Thus, it is clearly demonstrated through this work that the 5'-pixylated dideoxynucleoside phosphates bearing 3'-phosphodiester triethylammonium salt[3] (2) are suitable building blocks for solid phase synthesis of oligodeoxynucleotide fragments.

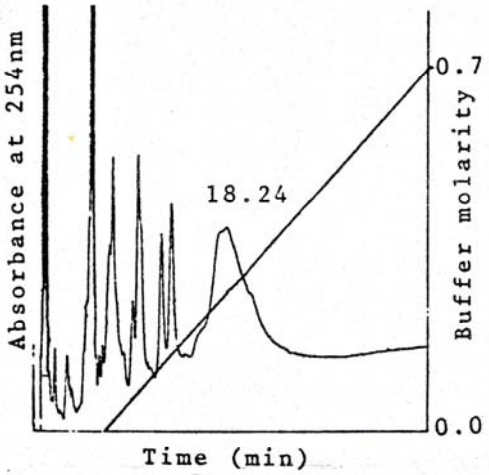


Figure 1

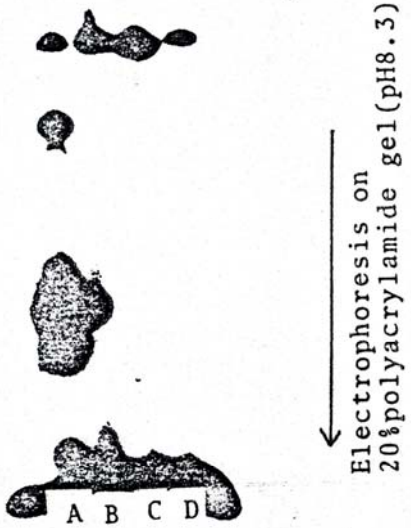
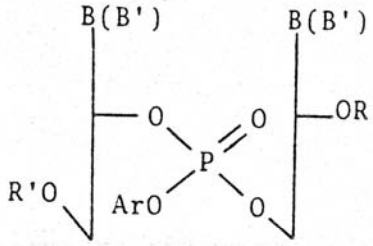
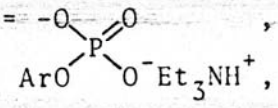


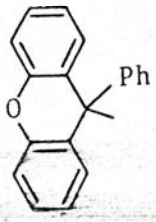
Figure 2



(I), R = H, R' = Pixyl

(2), R = 

R' = Pixyl



= Pixyl

Ar = o-ClPh

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References:

1. R. Crea and T. Horn, Nucleic Acid Res. 8, 2331 (1980) and references therein.
2. K. Miyoshi and K. Itakaura, Tetrahedron Letters, 3635 (1979).
3. J.B. Chattopadhyaya and C.B. Reese, J.C.S. Chem. Comm. 639 (1978)
   N. Balgobin, S. Josephson, J.B. Chattopadhyaya, submitted to Nucleic Acid Res.
4. 9-phenylxanthen-9-yl, used for 5'-protection, is abbreviated to Px (pixyl); 6-N-(m-chlorobenzoyl)-2'-deoxyadenosine, 2-N-(p-t-butylbenzoyl)-2'-deoxyguanosine and 4-N-benzoyl-2'-deoxycytidine are represented by A,G and C respectively. p and p denote internucleotide phosphotriester and 3'-terminal phosphodiester respectively with o-chlorophenyl protecting group.
5. C.B. Reese, R.C. Titmus and L. Yau, Tetrahedron Letters, 2727 (1978).
6. R. Frank and H. Köster, Nucleic Acid Res. 6, 2069 (1980).

The confirmation of the dodecamer sequence has been further established by an independent synthesis using a methodology[3] developed in solution chemistry (N. Balgobin and J.B. Chattopadhyaya, Unpublished result.)