Tetrahedron Letters,Vol.27,No.28,pp 3267-3270,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

CHEMICAL SYNTHESIS OF 5'-PHOSPHORYLATED OLIGODEOXYRIBONUCLEOTIDE ON A POLYMER SUPPORT

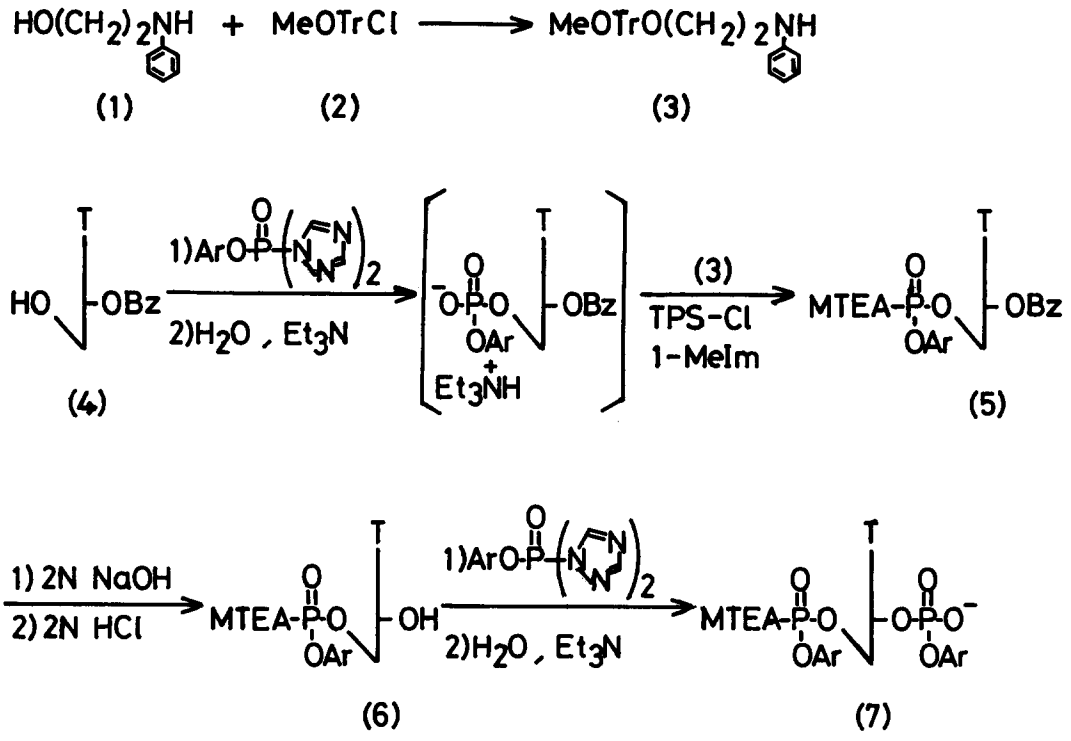
Toshiki Tanaka, Yasuki Yamada, and Morio Ikehara

Faculty of Pharmaceutical Sciences, Osaka University
1-6 Yamadaoka, Suita, Osaka 565, Japan Osaka 565, Japan

Summary
A highly hydrophobic N-methoxytrityloxyethylaniline(MTEA) was introduced for the protection of 5'-phosphate of thymidine. A MTEA group at the phosphodiester level is cleaved by aqueous acetic acid. By using MTEA group, 5'-phosphorylated hexadecadeoxyribonucleotide was rapidly synthesized on a polymer support.

The synthtic method of oligodeoxyribonucleotide with 5'-hydroxyl groups was already established either in solution or on solid support. A numerous oligodeoxyribonucleotide with defined sequence has been synthesized. On the polymer support synthesis, dimethoxytrityl group which is commonly used for the 5'-hydroxyl protection enable us to isolate the desired product with high purity very rapidly by the reversed phase column chromatography[1]. Such oligonucleotide was usually 5'-phosphorylated by the polynucleotide kinase and ATP to be used in the biological studies. Although the chemical synthesis of 5'-phosphorylated oligonucleotides was reported by others[2], the rapid purification of them was left out of consideration. Recently Uhlman et al. used 2-p-nitrophenylethyl group for the 5'-terminal phosphoryl protection and synthesized 5'-phosphorylated hexadeoxyribonucleotide on a polymer support[3]. In this paper we introduced the highly hydrophobic N-methoxytrityloxyethylanilino group to the 5'-phosphorylated thymidine and facilitated the rapid synthesis and purification of 5'-phosphorylated oligonucleotide on a polymer support.

First of all, 2-anilinoethanol (1) was reacted with 1.leq. of monomethoxytrityl chloride (2) in CH_2Cl_2 in the presence of N,N-diisopropylethylamine at r.t. for 30 min. After work up and separation by the silica gel column, N-methoxytrityloxyethylaniline (3) was obtained as a yellow oil in 98%



MeOTr = monomethoxytrityl Ar = o-chlorophenyl
MTEA = $MeOTrO(CH_2)_2N(C_6H_5)$-

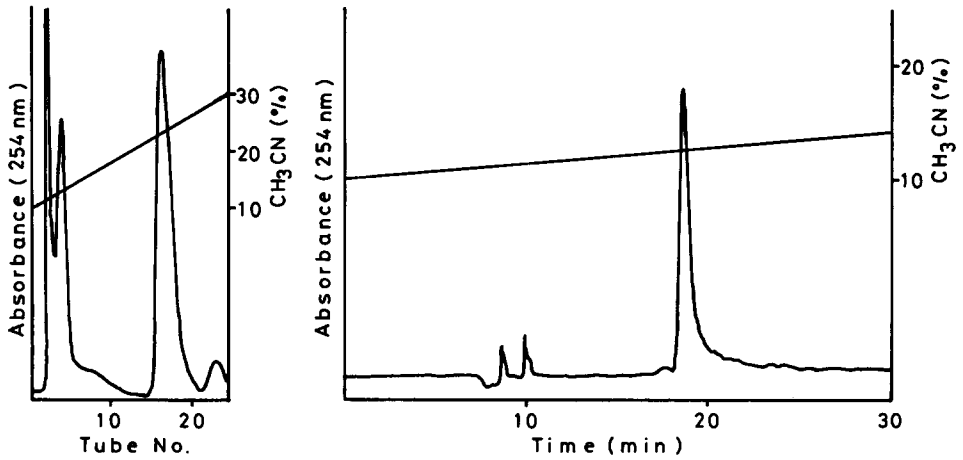


Figure 1. a) Isolation of (MTEA)-hexadecamer on the reversed phase C_{18} silica gel column. b) HPLC analysis of pTAAAACGACGGCCAGT on the reversed phase C_{18} silica gel column.

yield. To prepare compound (5), 3'-benzoylthymidine (4) was phosphorylated with 1.3eq of o-chlorophenylphophoroditriazolide, then hydrolyzed by the addition of H_2O. After the excess o-chlorophenylphosphate was removed by the extraction with H_2O, organic layer was evaporated and then condensed with 5eq of (3) using 4eq of 1,3,5-triisopropylbenzensulfonyl chloride (TPSCl) and 7eq of 1-methylimidazole (1-MeIm) in CH_2Cl_2 solution[4]. After 5hr at r.t.,the mixture was separated by the silica gel column and compound(5) was obtained as a white solid in 47% yield after precipitation into n-hexane from its solution of CH_2Cl_2.

For the removing all the protecting groups from (5), (5) was first treated with 0.5M N^1,N^1,N^3,N^3-tetramethylguanidinium syn-pyridine-2-aldoximate (TMG-PAO) in dioxane-pyridine-H_2O (4:2:1,v/v) at 60°C[5] for 12hr, then conc.NH_4OH at 60°C for another 5 hr to remove o-chlorophenyl and benzoyl protecting groups. The removal of MTEA group was accomplished by the treatment of 80% aq.AcOH at r.t. for 1hr to afford thymidine-5'-phosphate as an only product.

To prepare (7), (5) was treated with 2N NaOH in MeOH solution at 0°C for 10min. to remove the benzoyl group selectively then the mixture was neutrlized by the addition of 2N HCl. Usual work up and isolation by silica gel column gave (6) in 88% yield. (6) was then phosphorylated with o-chlorophenyl-phosphoroditriazolide and hydrolyzed by the addition of H_2O. The compound (7), which is used for the oligonucleotide synthesis by the phosphotriester method, was obtained as a white solid by the precipitation into n-hexane from its solution of CH_2Cl_2 in 92% yield.

In order to demonstrate the synthesis of oligodeoxyribonucleotide with 5'-phosphate, we synthesized the dimer, pTC, and the hexadecamer, pTAAAACGA-CGGCCAGT. For the synthesis of dimer, N-benzoyldeoxycytidine bound to a polystyrene support[6] was condensed with 6eq of (7) by 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) in pyridine for 40min. The resin was treated with 0.5M TMG-PAO at 60° for 12hr and conc NH_4OH at 60°C for 5hr to remove o-chlorophenyl and benzoyl protecting groups and to release the nucleotidic compounds from the resin. After the non-nucleotidic compound was removed by the reversed phase C_{18} column, the mixture was treated with 80% AcOH at r.t. for 1hr to yield unprotected dimer pTpC. The dimer, after isolation by the paper electrophoresis at pH 7.5, was subjected to digestion with bacterial alkaline phosphatase and venom phosphodiesterase to give T and pC in an equal amount. For the synthesis of hexadecamer, using 3μmol of thymidine bound to a polystyrene support, protected seven dimer AG, CC, GG, AC, CG, AA, AA[7] and one monomer (7) were successively condensed with MSNT according to the reaction cycle shown in the reference[8]. After the reactions, the resin was treated with

0.5M TMG-PAO at 60°C for 12hr and conc. NH_4OH at 60°C for 5hr. After the resin was removed, the mixture was subjected to the reversed phase C_{18} column and eluted with a linear gradient of CH_3CN in 50mM triethylammonium acetate at pH 7 (Figure 1). The hexadecamer having MTEA group at 5'-phosphoryl group was slowly eluted in a yield of $99A_{257}$ (0.62μmol, 21%). After the MTEA group was removed by 80% AcOH at r.t. for 1hr, 5'-phosphorylated hexadecamer was analyzed by the reversed phase C_{18} high pressure liquid chromatography. Purified 5'-phosphorylated hexadecamer was first treated with bacterial alkaline phosphatase, and then phosphorylated with $(\gamma^{32}P)$ ATP and T_4-polynucleotide kinase since 5'-phosphorylated hexadecamer was not labeled directly by $(\gamma^{32}P)$ ATP and kinase. The 5'-labeled hexadecamer was then confirmed in its nucleotide sequence by the mobility shift analysis[9].

In this paper, we applied the MTEA group for the protection of terminal phosphate. Since the MTEA group is highly hydrophobic, the oligonucleotide having MTEA group is easily isolated by the reversed phase column. Moreover, the removal of MTEA group is easily carried out by 80% AcOH. By using MTEA group, 5'-phosphorylated oligonucleotide can be synthesized very rapidly on a polymer support even in large scale. Application of this protecting group to the phosphite approach is under investigation.

References
1. M.D.Matteucci and M.H.Caruthers, Tetrahedron Lett., 21, 719 (1980).
2.a) E.Ohtsuka, T.Tanaka and M.Ikehara, Nucleic Acids Res., 7, 1283 (1979).
b) G.van der Marel, G.Veeneman and J.H.van Boom, Tetrahedron Lett., 22, 1463 (1981).
c) G.van der Marel, C.A.A.van Boeckel, G.Wille and J.H.van Boom, Nucleic Acids Res., 10, 2337 (1982).
3. E.Uhlman and J.Engels, Tetrahedron Lett., 27, 1023 (1986).
4. V.A.Efimov, S.V.Reverdatto and O.G.Chakhmakhcheva, Tetrahedron Lett., 23, 961 (1982).
5. T.P.Patel, M.A.Chauncey, T.A.Millican, C.C.Bose and M.A.W.Eaton, Nucleic Acids Res., 12, 6853 (1984).
6. H.Ito, Y.Ike, S.Ikuta and K.Itakura, Nucleic Acids Res., 10, 1755 (1980).
7. 5'-hydroxyl, heterocyclic amino and phosphate groups were protected by dimethoxytrityl, benzoyl or isobutyryl and o-chlorophenyl groups respectively.
8. T.Tanaka and T.Oishi, Chem. Pharm. Bull., 33, 5178 (1985).
9. E.Jay, R.Bambara, P.Padmanabhan and R.Wu, Nucleic Acids Res., 1, 331 (1974).

(Received in Japan 7 April 1986)