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TRITYLOXYETHYLAMINO GROUP FOR THE PROTECTION OF PHOSPHORYL GROUP IN OLIGONUCLEOTIDE SYNTHESIS

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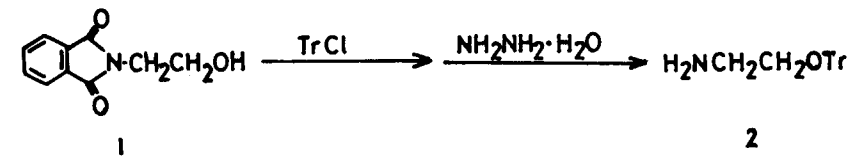
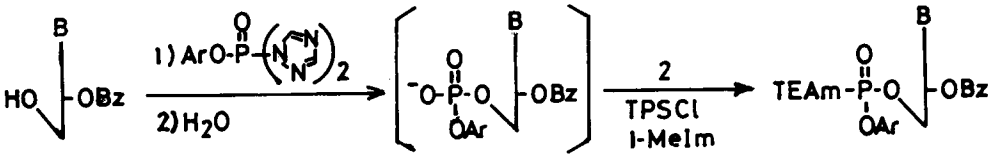
Summary
Trityloxyethylamino group was used for the protection of 5'-phosphoryl group of deoxyribonucleoside. This group is not only protecting group of phosphate also the intermediate for the synthesis of d-ppA and d-pppA.

The synthesis of deoxyoligonucleotide with a defined sequence is accomplished very rapidly by either phosphotriester or phosphite-triester approach on a polymer support. As the chemically synthesized oligonucleotide has generally 5'-hydroxyl group, it is phosphorylated by the polynucleotide kinase and ATP at 5'-end for biological studies[1].

We tried to synthesize oligodeoxynucleotide with 5'-phosphoryl group. Recently we used N-methoxytrityloxyethylanilino group(MTEA)[2] for the protection of phosphoryl group. By using MTEA in conjunction with o-chlorophenyl group for the protection of 5'-phosphoryl group, we have succeeded in the rapid synthesis and purification of hexadecadeoxyribonucleotide with 5'-phosphoryl group by the phosphotriester approach on a polystyrene support. In the course of the deblocking step,however,o-chlorophenyl group on the 5'-terminal phosphate was not removed by 0.5M N^1,N^1,N^3,N^3-tetramethylguanidinium syn-pyridine-2-aldoximate (TMG-PAO)[4] at room temperature overnight or conc. ammonia at 55°C overnight though o-chlorophenyl group on the internal phosphate was completely removed by these treatments. However,it was found that o-chlorophenyl group was removed by the treatment with 0.5M TMG-PAO at 50°C overnight [5]. In order to find another lipophilic phosphoryl protecting group which is removed by mild basic treatment, we prepared trityloxyethylamine as follow. N-Hydroxyethylphthalimide (1) was treated with 1.2eq. of trityl chloride in CH_2Cl_2 using triethylamine as a catalyst. After the completion of the reaction was confirmed by silica gel TLC(CH_2Cl_2,R_f 0.20→0.72),hydrazine hydrate (30 eq.)was added to the mixture without purification. After the completion of the reaction was confirmed by TLC (CH_2Cl_2, Rf=0.72→0.10),the mixture was separated by the silica gel column chromatography to yield trityloxyethylamine (2) in a yield of 82% as a white solid [6].

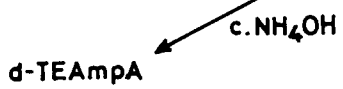


1 2

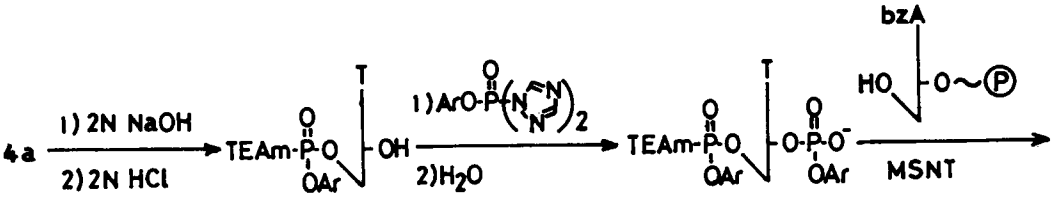


3a B = T
b B = bzA
c B = bzC
d B = ibG

4



d-TEAmpA
5



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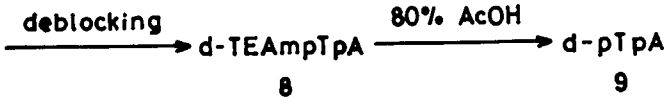
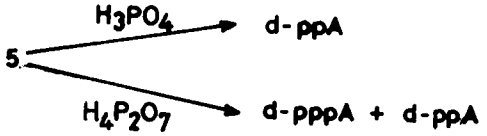


8 9

Tr = trityl Ar = o-chlorophenyl
TEAm = $TrO(CH_2)_2NH$-



To synthesize the compound (4, a-d),protected deoxynucleoside with free 5'-hydroxyl (3, a-d) was first phosphorylated with 1.5eq. of o-chlorophenyl-phosphoroditriazolide in CH_2Cl_2 solution. After hydrolysis by adding water then extraction with CH_2Cl_2,phosphorylated intermediate was condensed with trityloxyethylamine (2) (2 eq) using 1,3,5-triisopropylbenzenesulfonyl chloride (TPSCl) (4eq) and 1-methylimidazole (7 eq) [7] in CH_2Cl_2 for 1hr at room temperature. After usual work up, the mixture was purified by the silica gel column to afford compound (4, a-d) in yields of 57, 69, 83 and 61%, respectively, as white solid after the precipitation into n-hexane from its solution of CH_2Cl_2.

In order to remove protecting groups, the compound (4b) was treated with conc. ammonia at 50°C for 3hr. It is found that benzoyl and o-chlorophenyl groups were completely removed by this treatment and deoxyadenosine-5'-trityl-oxyethylaminophosphate (5) was obtained as a sole product. The removal of trityloxyethylamino (TEAm) group was accomplished by the treatment of 80% aq. AcOH for 2hr at room temperature to give deoxyadenosine-5'-phosphate.

The compound (4a) was treated with 2N NaOH to generate a free 3'-hydroxyl in a yield of 75%. (6) was then phosphorylated with o-chlorophenylphosphor-ditriazolide followed by the hydrolysis with water to give compound (7) in 86% yield. The 5'-hydroxyl group of N-benzoyldeoxyadenosine bound to a polystyrene support was condensed with 3eq of the diphosphorylated compound (7) using 1-mesityrenesulfonyl-3-nitro-1,2,4-triazole (MSNT) in pyridine. After 45 min at room temperature, the resin was treated with 0.5 M TMG-PAO in dioxane-pyridine-H_2O (4:2:1, v/v) for 16 hr at room temperature then c. NH_4OH at 60° for 10 hr to remove the benzoyl and o-chlorophenyl protecting groups and to release the nucleotidic compounds from the resin. The mixture was separated by a reversed phase (C_{18}) silica gel column and afforded dimer TEAmpTpA (8) in 80% yield. Then TEAm group was removed by the treatment with 80% aq. AcOH at room temperature for 3 hr to give pTpA (9) in 89% yield [8] after the separation by the paper electrophoresis (50mM TEAB, pH 7.5). This dimer was subjected to the enzyme digestion by venom phosphodiesterase to give pT and pA in an equal amount.

It is considered[9] that trityloxyethylamino group can be used as the protecting group of intermediate in synthesis of nucleoside di or triphosphate. The compound (5) (7μmol) (tri-n-butylammonium salt) and phosphoric acid (tri-n-butylammonium salt)(20eq), after co-evaporation with pyridine, then toluene in separated vessels, were mixed in DMF (0.4ml). After 3days at 40°C, the mixture was diluted with H_2O(1ml), washed with ether (8ml) and separated by the Sephadex A-25 column using a linear gradient of triethylammonium bicarbonate (TEAB, pH 7.5) (0M→0.6M). Deoxyadenosine 5'-diphosphate was eluted at 0.3M TEAB concentration and obtained in 45% yield. With the same procedure, tri-n-butylammonium salt of the compound (5) and pyrophosphoric acid (20eq) was reacted in DMF solution. After 12 hr at 40°C, deoxyadenosine-5'-triphosphate (23%) and deoxyadenosine-5'-diphosphate

(21%) which came from the dephosphorylation of the former product, were obtained after the separation by Sephadex A-25 column chromatography.

In this report, we prepared trityloxyethyamine and used it for the protection of phosphate. Trityloxyethylamine can be easily introduced to phosphoryl group with TPSCl and 1-methylimidazole. Since trityloxyethylamino (TEAm) group is highly lipophilic, the oligonucleotide with this group will be purified easily by the reversed phase column. Moreover this protecting group is removed from phosphate by 80% AcOH treatment to afford nucleoside-5'-monophosphate. Finally we showed the synthesis of deoxyadenosine-5'-di or triphosphate. In conclusion, TEAm group can be used not only for the protection of phosphoryl group also for preparing nucleoside di- or triphosphate. The synthesis of pppAp(2'-5')Ap(2'-5')A, which has antitumor activity, by using TEAm group is now in progress.

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