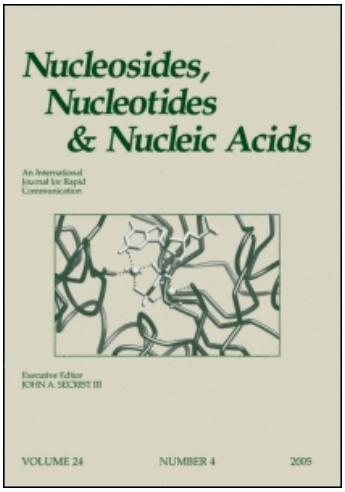

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Solution Phase Synthesis of an Oligodeoxyribonucleotide Phosphorothioate for Therapeutic Applications

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Abstract: Solution phase synthesis of an oligodeoxyribonucleotide phosphorothioate octamer (5'-TTGGGGTT) using phosphorothioate triester method is reported.

Recently, considerable attention has been focused on the applications of oligonucleotides as therapeutic agents.[1-4] Although, initial interest in oligonucleotides was focused on their interaction with nucleic acid receptors, interest has broadened to include the use of oligonucleotides as ligands to interact with non-nucleic acid receptors like proteins as well. Thus, the creation of combinatorial libraries of oligonucleotides containing natural and unnatural motifs has greatly facilitated the exploration of these potential interactions for therapeutic purposes.[5-7]

The oligodeoxyribonucleotide phosphorothioate TTGGGGTT (ISIS 5320) is a potent inhibitor of HIV infection *in vitro*. The compound was identified by combinatorial screening of a library of all possible octanucleotide sequences.[8] This octanucleotide forms a parallel stranded, tetrameric guanosine-quartet (G-quartet) structure which specifically binds to the HIV envelope glycoprotein (gp 120) and inhibits both cell-to-cell and virus-to-cell infection at submicromolar concentrations. This tetramer inhibits the infection of laboratory-derived isolates of HIV-1 and HIV-2 in a variety of phenotypically distinct, established human cell lines and a panel of biologically diverse clinical isolates in fresh human peripheral blood lymphocytes and macrophages. The compound was also active against all drug-resistance virus isolates tested. In combination with AZT, it exhibits additive to slightly synergistic anti-HIV activity. Cell-based mechanism of action studies demonstrate that the compound inhibits the binding of infectious virus and virus-infected cells to uninfected target cells by binding to the cationic V3 loop of the envelope glycoprotein.[9]

This potential drug candidate for the treatment of AIDS is presently under pre-clinical trials. Very large quantities (several hundred kilograms) of this drug are needed for clinical trials and several folds more to meet market demands. Thus a key issue is the selection of a method for manufacturing the compound in multi-kilogram quantities to fully support clinical trials in the AIDS population at an acceptable cost.

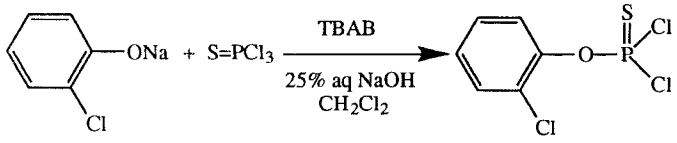
Solution-phase chemical synthesis of nucleic acids has significant potential in drug synthesis and is particularly appropriate for large-scale preparation of short sequence oligonucleotides.[10-11] Low cost reagents and operational simplicity allowing large scale, reproducible syntheses are important practical considerations. As a part of our on-going investigation on the solution phase synthesis, we were interested in the phosphotriester approach for the large scale synthesis of this octamer. Although there are several publications on the solution phase synthesis, there is no report on the synthesis of oligonucleotides in multigram quantities. Thus an industrial production of this magnitude is without precedent in oligonucleotide chemistry and because of its commercial value, attempts to explore relative merits of its economical synthesis warrants careful consideration.

Method of Synthesis

Among the three methods of coupling chemistries, viz. phosphoramidite, H-phosphonate and phosphotriester,[12-14] the last approach is more suited for solution phase synthesis of oligonucleotides.

Synthesis of Ary Phosphorodichloridothioate

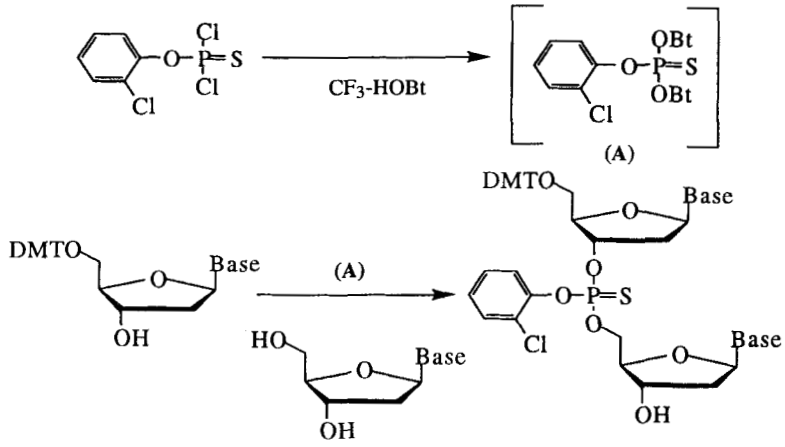
The reported procedures for the synthesis of *o*-chlorophenylphosphorodichlo ridothioate are not convenient for large scale synthesis. They involve either using liquid sulfur trioxide or refluxing at high temperatures affording low yields of the desired product. A convenient two-phase synthesis of the title compound was carried out in our laboratory using a phase transfer catalyst.[15]



Treatment *o*-chlorophenol in 25% aqueous sodium hydroxide with 4 equivalents of thiophosphoryl chloride in dichloromethane in presence of catalytic

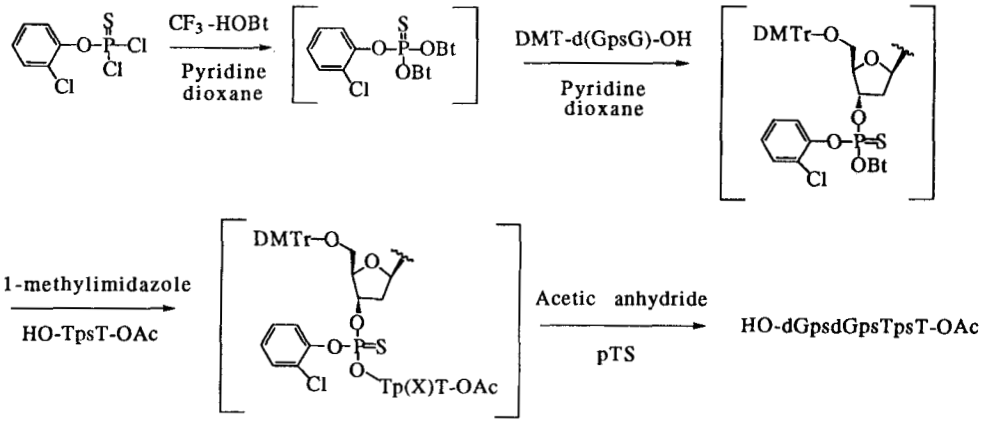
amount of *tetra*-butylammonium bromide at room temperature for 8 h gave *o*-chlorophenylphosphorodichloridothioate in 96% yield after distillation. We were easily able to synthesize several hundred grams of this compound.

Synthesis of Dimer Blocks



The efficiency of coupling utilizing various reagents were investigated in the synthesis of homodimers of T and dG. We observed that 1-hydroxy-6-(trifluoro methyl)benzotriazole afforded higher yields as compared to other reagents.[16]

Oligomerization of Dimers

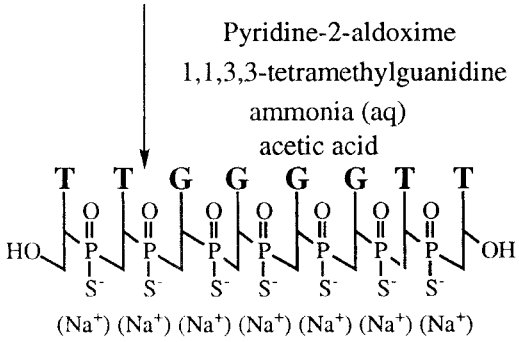


Chain Elongation

5'-HO-d(GpsGpsTpsT)-OAc ---> 5'-HO-d(GpsGpsGpsGpsTpsT)-OAc ---> 5'-DMT-d(TpsTpsGpsGpsGpsGpsTpsT)-OAc (fully protected octamer).

Deprotection and Purification of Octamer

5'-DMT-d(TpsTpsGpsGpsGpsGpsTpsT)-OAc



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