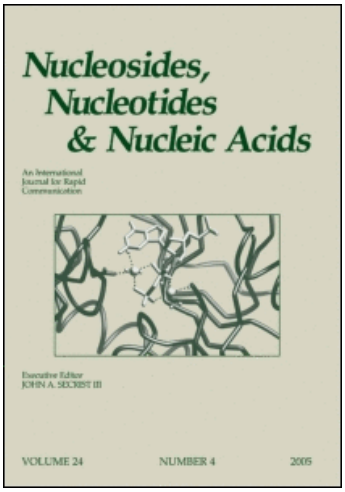

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A Simple Method For Introducing -SH/COOH Group AT 5'-OH End Of Oligonucleotide

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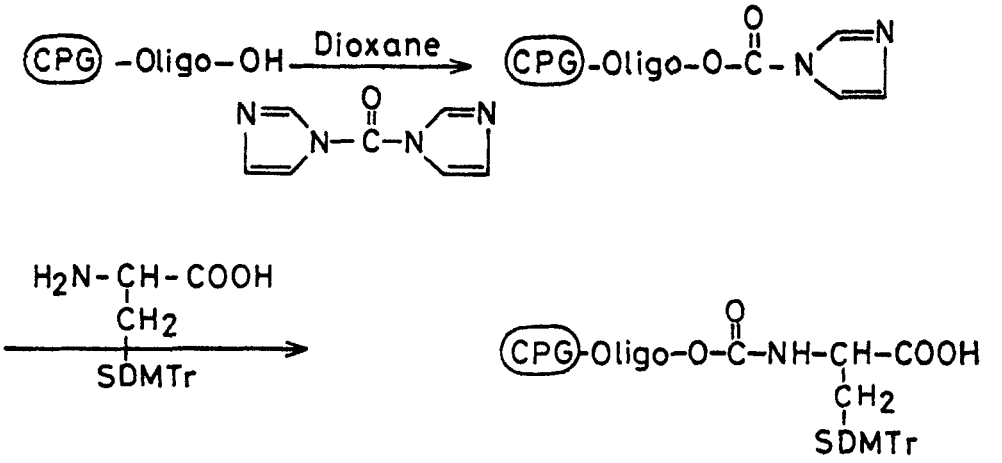
Abstract: A method for introducing a -SH group at the 5'-end of the oligonucleotide using S-Trityl-L-Cysteine has been developed.Carbonyl diimidazole was used for activating the 5'-OH group.

Several methods have been described for introducing thiol group at the 5'OH end of oligonucleotides.(1,2,3).However,all the methods developed so far make direct or indirect use of a phosphoramidite.Here,we describe a simple method for introducing a thiol group which does not require a phosphoramidite directly or indirectly.Interestingly our method also provides a carboxyl group along with the thiol group at the 5'-OH of the oligonucleotide.The reaction of oligonucleotide with carbonyl diimidazole and S-Trityl-L-Cysteine is shown in scheme-1.

An oligonucleotide d(TACGTTGCCTG) was synthesised as described in Pharmacia Gene Assembler manual.Polymer support was dried under vacuum over night.The 5'-OH of the oligonucleotide was activated by carbonyl diimidazole as described by Ramachandran et.al(4). S-Trityl-L-Cysteine was prepared as described in literature



Scheme-1

(5).200 mM Of the S-trityl cysteine solution prepared in dioxane: water (9:1) was added to the activated oligonucleotide.The reactant were allowed to react for 30 minutes under argon atmosphere.Excess of the reagent was washed off with dry dioxane (2x5).Subsequently the polymer support was washed with methanol and finally dried in the descicator overnight.The 1 mg of the polymer supports was treated with 2% dichloroacetic acid in dichloroethane for 2 minutes. Absorbance of the orange colour of $DMTr^+$ thus obtained was read at 498 nm,which showed that the reaction was 60%.This may be due to the partial deprotection of the $DMTr^+$ group.The remaining polymer support was treated with 25% aqueous ammonia for six hours at 60^0 C.The reaction vial was chilled in a ice box for 30 minutes and the polymer support was filtered off.The ammonia solution thus obtained was evaporated.The oligonucleotide was run on 8% PAGE-7M Urea to compare the electrophoretic mobility with the unlabeled oligonucleotide.The labeled oligonucleotide moved a little slower than the unlabeled oligonucleotide as was seen by the UV-shadowing.The oligonucleotide thus obtained was purified by passing through G-10 Sepadex column.The purified

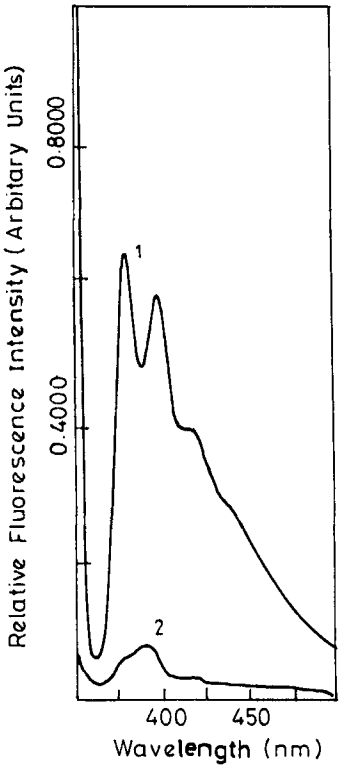


Figure-1.Fluoresence spectra (1)oligonucleotide-pyrenyl conjugate;

(2)N-(3-Pyrenyl)maleimide at a concentration of 3 nmole/ml.

oligonucleotide was reduced with $AgNO_3$ /DTT as described by Connolly et.al.The oligonucleotide was further desalted by passing through a G-10 Sephadex column.The thiol groups were 95% as was determined by Ellman's reagent test (6).

Finally N-(3-pyrenyl)maleimide was reacted with -SH on oligonucleotide (7).The 1 OD A 260 nm of -SH on oligonucleotide was dissolved in 250 µl of phosphate buffer(pH 7.5).1 mg of N(1-pyrene) maleimide dissolved in 250 µl of 50% ethanol-acetone was added to the oligonucleotide solution.The reaction was allowed to proceed for two hours at room temperature in dark.The resulting solution was reduced to 100 µl.The excess of the N-(3-pyrenyl)maleimide was removed by passing the reaction

mixture through a G-10 Sephadex column.Fluorescence spectrum of the pyrenyl labeled oligonucleotide obtained by excitation at 343 nm is shown in Fig-1.The spectrum shows peaks at 380 nm and 396 nm.The spectrum of the N-(3-Pyrenyl)-maleimide of the same concentration is also shown in Fig.-1. A comparison of the spectra shows that only the conjugate shows intense fluorescence at this concentration.

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