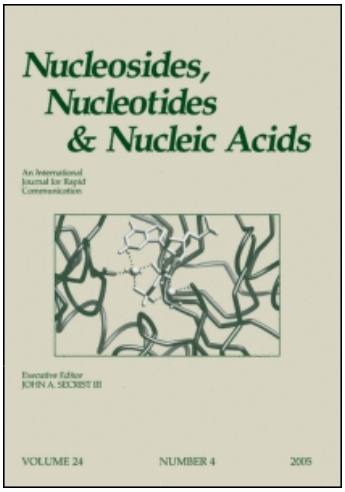

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A Simple Method for Introducing -SH Group at 5'OH Terminus of Oligonucleotide

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A Simple Method For Introducing -SH Group AT 5'OH Terminus Of Oligonucleotide

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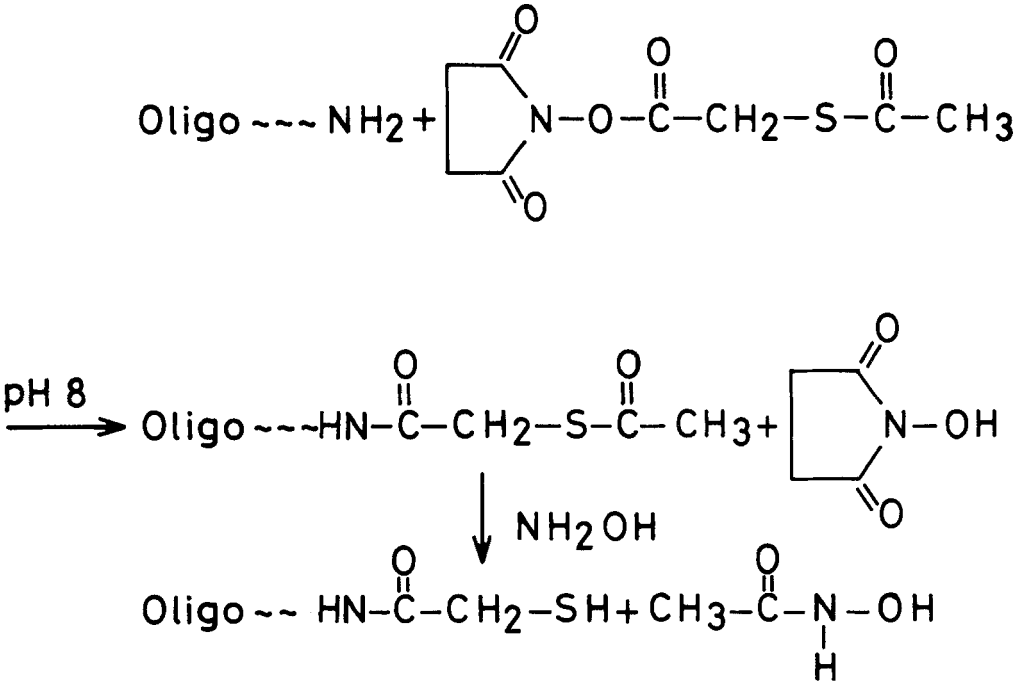
Abstract A simple method for introducing a -SH group at 5'-end of oligonucleotide using a commercially available reagent,N-succinimidyl-S-acetyl-thioacetate(SATA) is reported.

Several methods have been developed for introducing the -SH group at 5' OH of the oligonucleotide(1).A recent reported method make use of the commercially available reagent SPDP(2,7).The reaction was quantitative in the presence of DMAP.However, to get free -SH, the modified oligonucleotide was treated with DTT.Therefore the excess DTT has to be removed carefully before proceeding for the conjugation.Here we report a new reagent which has earlier been used for the introduction of -SH group into the proteins(4) and does not involve the use of another thiol such as DTT, for reduction. The reaction of SATA with aminolinked oligonucleotide is shown in scheme-1.



Scheme-1

A oligonucleotide d(GCAGTCAC) was synthesised over Pharmacia Gene Assembler.Amino group was introduced as described by Ramachandran using carbonyl diimidazole and hexamethylenediamine (3).The NH_2-on oligonucleotide was treated with 25-26% ammonia solution at 60^0C for 6 hours.The ammonia solution was evaporated in speed vac concentrator.Crude material was passed through a G-10 Sephadex column to remove the non nucleoside material.The oligonucleotide was dissolved in 250 µl of the sodium phosphate buffer(pH 8) .Thereafter 2 mg of the SATA dissolved in 250 µl of the DMF was added . The reaction was allowed to proceed for three hours at 37^0C.Subsequently the reaction mixture was completely dried in speed vac and redissolved in 100 µl of autoclaved water,and passed through a G-10 Sephadex column to remove the unreacted SATA. The purified oligonucleotide thus obtained was treated with 100 µl of 50 mM hydroxylamine hydrochloride ,2.5 mM EDTA, pH 7.25 for 2 hours. The thiol groups were >95% as

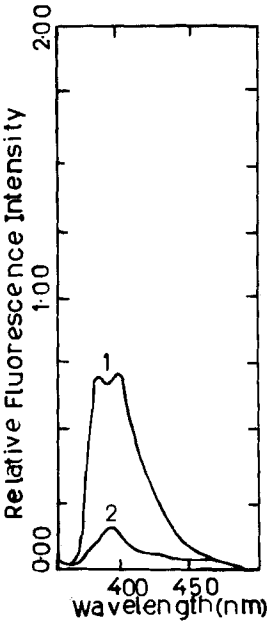


Figure-1.Fluorescence spectra (1)oligonucleotide-Pyrenyl conjugate; (2)N-(3-Pyrenyl)maleimide at a concentration of 3 nmole/ml.

determined by Ellman's reagent test(5).Finally modified oligonucleotide was reacted with N-(3-Pyrenyl) maleimide(6). 2 OD A260 nm of the SH- on oligonucleotide was dissolved in 250 µl of the phosphate buffer(pH 8).To this oligonucleotide solution, 1 mg of N-(1-pyrene)maleimide dissolved in 250 µl of 50% ethanol-acetone was added.The reaction mixture was allowed to stand at 37^0C in dark over night.The resulting solution was evaporated to dryness.The excess of the N-(3-pyrenyl)maleimide was removed by passing the reaction mixture through a G-10 Sephadex column.The fluorescence spectrum of the pyrenyl labeled oligonucleotide obtained by excitation at 343 nm showed peaks at 380 nm and 396 nm. is shown in Fig.-1.The spectrum of the N-(3-Pyrenyl)-maleimide of the same concentration is also shown in Fig.-1.A comparison of spectra

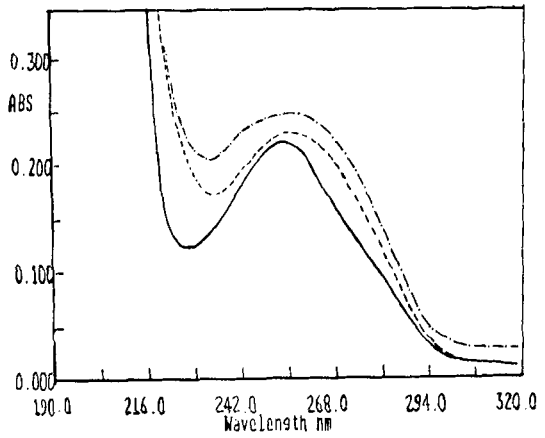


Figure-2.UV spectra of (——) pure oligonucleotide;(---)hydroxylamine treated oligonucleotide; (-·-) hydroxylamine treated pyrenyl maleimide labeled oligonucleotide.

suggest that only the conjugate shows intense fluorescence at this concentration.The UV spectra of pure oligonucleotide,hydroxylamine treated oligonucleotide and hydroxylamine treated pyrenylmaleimide labeled oligonucleotide shown in Fig.-2 show λ max at 254 nm suggesting that there is no change in structure of oligonucleotide after hydroxyamine treatment and after labelling with pyrenyl maleimide.

References

1.Clontech -DNA modification reagents for the use in automated synthesis manual and reference therein.
2.Gaur,R.,Sharma,S.and Gupta,K.C. Nucleic Acids Res.17 4404 (1989).
3.Wachter,L.,Jabloski,J.L.and Ramachandra,K.L.Nucleic Acids Res.14 7985-7994 (1986).

4.Duncan,R.J.S.,Weston,P.D.and Wrigglesworth, R.Analytical Biochemistry **132** 68-73 (1983).
5.Pierce ImmunoTechnology Catalog & Handbook.
6.Wu,C.W.,Yarbrough,L.R. and Hsinchwu,F.Y.Biochemistry **15** 2863-2868 (1976).
7.Gaur,R.K.Nucleoside & Nucleotides **10** ,895 (1991)

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