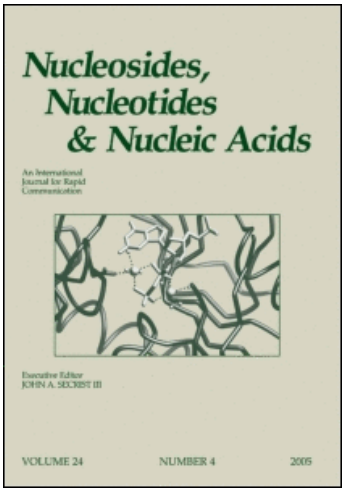

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Synthesis and Antibody Mediated Detection of 2,4-Dinitrophenyl (DNP) Labelled Oligonucleotides

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SYNTHESIS AND ANTIBODY MEDIATED DETECTION OF 2,4-DINITROPHENYL (DNP) LABELLED OLIGONUCLEOTIDES.

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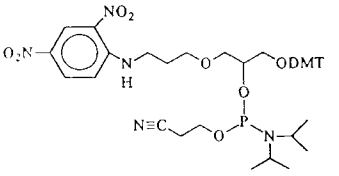
Abstract: Different DNP phosphoramidites based on non-nucleoside and nucleoside backbone molecules are developed and used in the multiple labelling of oligonucleotides during the solid phase synthesis. It is demonstrated that the antibody mediated detection of DNP labelled oligonucleotides is comparable to that of digoxigenin, biotin and fluorescein.

INTRODUCTION

The use of non-radioactively labelled DNA/RNA probes for a variety of applications including the diagnosis of diseases based on in situ hybridisation techniques is becoming increasingly popular. This approach offers several advantages over the conventional radioactive labelling methods, since radioactive labels are hazardous, expensive and have a limited shelf life. Although the currently available non-radioactive labels such as biotin,[1,2] digoxigenin[3] or fluorescein are widely used they have some limitations. There is a real need for an alternative non-radioactive labelling system which can be used for a variety of applications.

RESULTS AND DISCUSSION

The prime objective of this study was to develop a non-radioactive labelling system using the hapten 2,4-dinitrophenyl (DNP). This label is small, chemically simple, antigenic and is not found *in vivo*. Recently it has been demonstrated[4] that the glycerol based DNP monomer (1) is suitable for multiple additions at the



(1)

5'-end of an oligonucleotide and that the use of 3 DNP labels was of better sensitivity than 1 label and at least as good as 5 labels. Colorimetric detection of hybridised DIG-, DNP-, and fluorescein-labelled

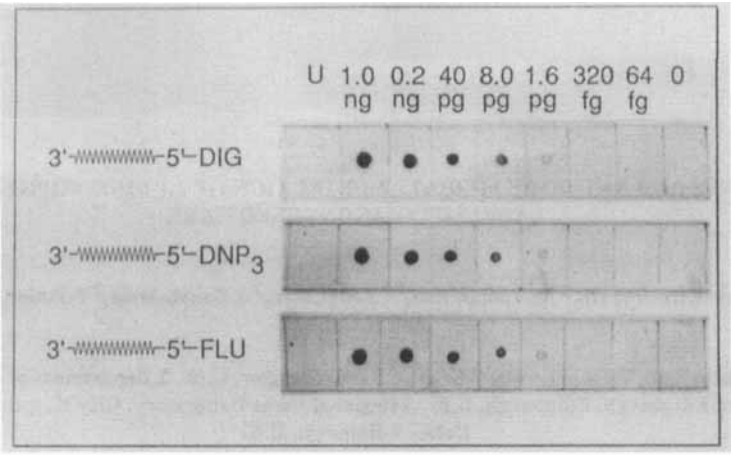


FIGURE 1 Colorimetric detection of hybridised DIG-, DNP-, and fluorescein-labelled 24mer probes

24mer probes (FIGURE 1) using the corresponding antibody alkaline phosphatase conjugates demonstrated[5] that the sensitivity obtained when using oligonucleotides labelled with glycerol DNP was comparable to that obtained with DIG and fluorescein labelled oligonucleotides.

In another experiment[6] DNP- and DIG- labelled oligonucleotides were compared in the detection of human parvovirus B19. The results obtained indicate that these two systems are equivalent, with detection sensitivity down to 10fg of B19 DNA. Oligonucleotides labelled with glycerol DNP have also been used successfully for detecting PCR products.[7]

After demonstrating the use of DNP-labelled oligonucleotides, using the glycerol based backbone molecule, we focused our attention on the synthesis (SCHEME 1) of a nucleoside based DNP phosphoramidite monomer. The aim was to develop a DNP-label which could be incorporated within the oligonucleotide during its solid phase synthesis and could be used in conjunction with the glycerol based DNP-label. This would allow multiple DNP-labelling of synthetic oligonucleotides which is difficult with the fluorescein system and not possible with the DIG system. It is important to note that in these monomers (2) and (3) both hydrogen bond donor-acceptor sites are available for Watson-Crick base pairing and that the triple bond situated α to C-5 ensures optimal hybridisation characteristics and avoids the possibility of geometric isomers which are present in the analagous C-5 propenyl monomer. In order to determine the optimum length of linker arm we have synthesised two DNP labelled phosphoramidites (2) and (3) differing only in the length of linker arm used. Evaluation of the use of (2) and (3) in the synthesis of a 39-mer and the corresponding hybridisation experiments (FIGURE 3) indicate that greater sensitivity can be achieved with the longer linker arm. We therefore used only monomer (2) in subsequent experiments.

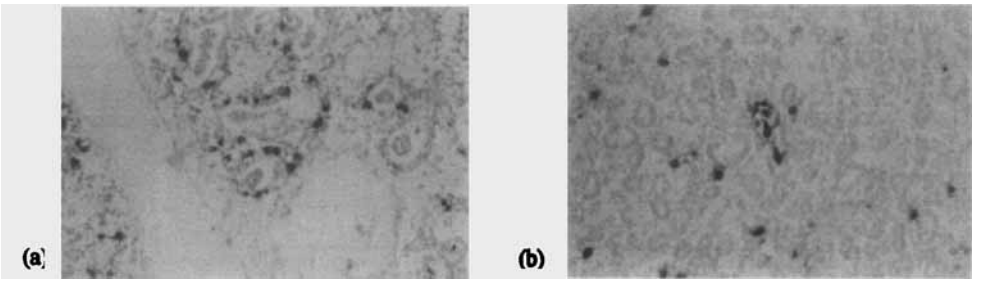
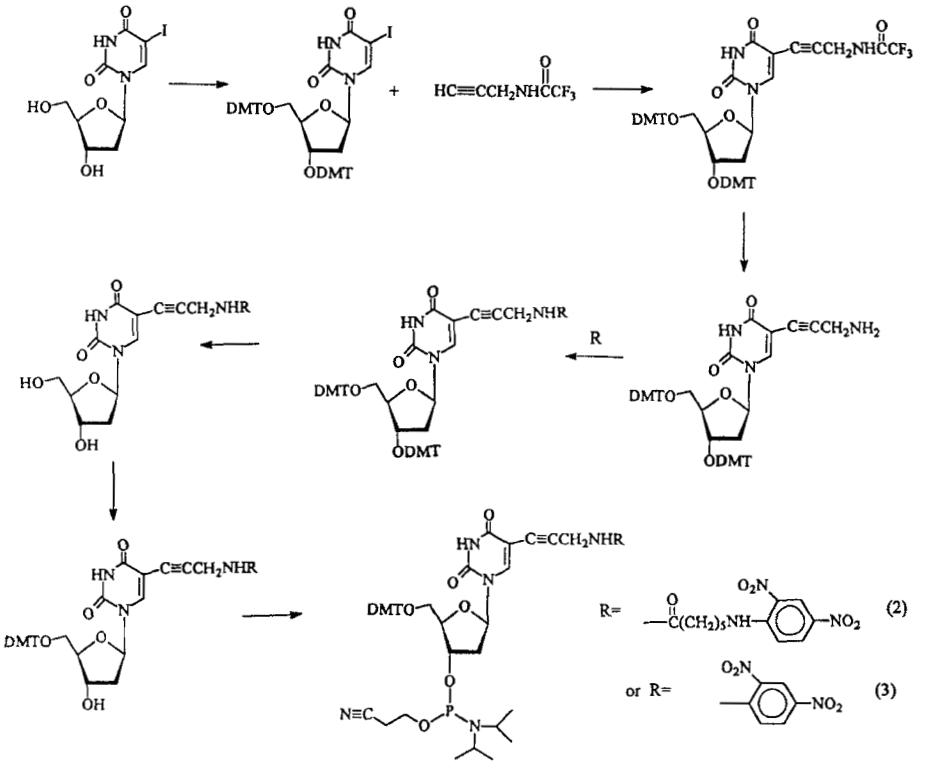


FIGURE 2 Colorimetric detection of *in situ* hybridisation for the detection of parvovirus in (a) the lung tissue and (b) the tracheal gland tissue from a case of fetal hydrops using a pool of 5 DNP -labelled oligonucleotides and anti-DNP antibody alkaline phosphatase conjugate.



SCHEME 1

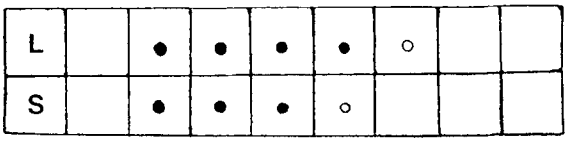


FIGURE 3 Colorimetric detection using DNP-labelled 39-mers. L was synthesised using monomer (2) with the linker arm and S using monomer (3).

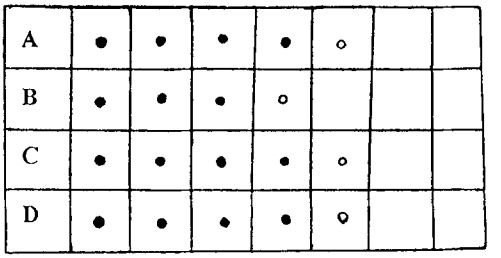


FIGURE 4 Dot blots show that the combination of terminal DNPs and internal DNPs increases the level of sensitivity obtainable. A=40mer 3 dU DNP, B=44mer 3 glycerol DNP, C=111mer 15 dU DNP and 6 glycerol DNP, D=36mer 6 dU DNP and 5 glycerol DNP

The dot blots of DNP-labelled probes (FIGURE 4) indicate that the dU DNP system is marginally better than the glycerol DNP system and that the detection limits are further improved when the dU DNP label is used in conjunction with the glycerol DNP label.

We have synthesised the corresponding dU DNP triphosphate and are currently evaluating its performance in PCR and related applications.

It has been demonstrated that the DNP labelling system, especially when glycerol DNP and dU DNP are used together, is a more attractive alternative to the DIG, biotin and fluorescein-labelling systems.

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