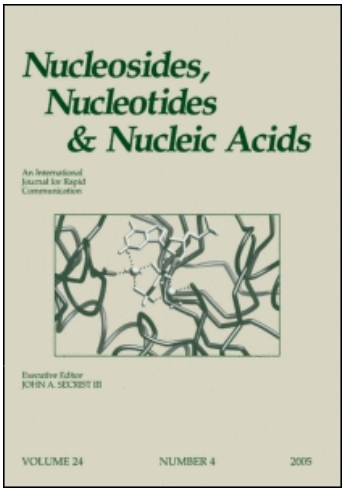


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A New Multiple Labelling Method for Synthetic Oligonucleotides and Increased Sensitivity in DNA Detection

Konrad Misiura[a]; Ian Durrant[b]; Michael R. Evans[b]; Michael J. Gait[a]
[a] MRC Laboratory of Molecular Biology, Cambridge, UK [b] Amersham International plc, Pollards Wood Laboratories, Buckinghamshire, UK

To cite this Article Misiura, Konrad , Durrant, Ian , Evans, Michael R. and Gait, Michael J.(1991) 'A New Multiple Labelling Method for Synthetic Oligonucleotides and Increased Sensitivity in DNA Detection', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 671 — 672
To link to this Article: DOI: 10.1080/07328319108046568
URL: http://dx.doi.org/10.1080/07328319108046568

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 671-672 (1991)

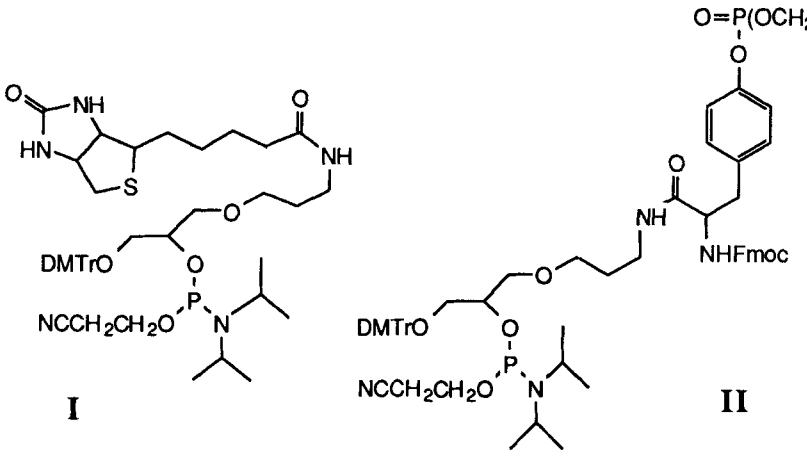
A NEW MULTIPLE LABELLING METHOD FOR SYNTHETIC OLIGONUCLEOTIDES AND INCREASED SENSITIVITY IN DNA DETECTION

Konrad Misiura*, Ian Durrant†, Michael R. Evans†, and Michael J. Gait*
*MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK
and † Amersham International plc, Pollards Wood Laboratories, Nightingales Lane, Chalfont St Giles, Buckinghamshire, HP8 4SP, UK.

ABSTRACT. Non-nucleosidic phosphoramidite units suitable for use on commercial DNA Synthesis machines have been prepared for direct incorporation of biotins or phosphotyrosines at multiple sites in synthetic oligonucleotides.

Synthetic oligonucleotides labelled with non-radioactive reporter groups are finding increasing applications as hybridisation probes and diagnostic reagents for the detection of DNA and RNA. However, use of singly labelled oligonucleotides sometimes leads to insufficient sensitivity or signal strength. Hitherto, methods for incorporation of multiple reporter groups (eg biotin) into synthetic oligonucleotides have relied either on post-assembly functionalisation of oligonucleotides containing multiple amino groups [1] or incorporation of a 4-N-(6-N-biotinylaminohexyl)-2'-deoxycytidine derivative [2].

We now report the synthesis of simple, non-nucleosidic phosphoramidite units suitable for the incorporation of reporter groups at multiple sites in a synthetic oligonucleotide. The units are based on a 3-carbon glyceryl backbone where the reporter group (biotin I or the new



I II

reporter group phosphotyrosine **II**) is attached to the 2-O-position through a 3-aminopropyl spacer. The synthesis of these reagents is reported elsewhere[3].

The phosphoramidite derivatives **I** and **II** were used in solid-phase oligonucleotide synthesis on an ABI 380B Synthesiser in place of a normal nucleoside phosphoramidite. Coupling reactions were identical to standard conditions except that the concentration in acetonitrile was 0.2M and the coupling time was increased to 300 secs.

Eight 17-mer oligodeoxyribonucleotides were assembled each corresponding to the universal M13-sequencing primer but followed by a number of extra cycles of coupling (1, 2, 4 or 8) using either biotin amidite **I** or phosphotyrosine amidite **II** . After deprotection with concentrated ammonia followed by acidic removal of terminal dimethoxytrityl groups, the oligonucleotides were purified by either reversed phase hplc (biotin) or ion exchange hplc or polyacrylamide gel electrophoresis (phosphotyrosine).

Oligonucleotides containing different numbers of biotins or phosphotyrosines at the 5'-end were compared as to the sensitivities of detection of single-stranded M13 DNA immobilised on nitrocellulose membranes. Detection of biotin involved use of a mouse anti-biotin monoclonal antibody followed by an anti-mouse IgG coupled to alkaline phosphatase and a colorimetric endpoint. A 5-10 fold increase in sensitivity of detection was obtained with a 5'-tail of 8 biotins. Improved quantitation of signal strength was acheived by use of Enhanced Chemiluminescence and detection with a CCD camera[4]. Here the secondary antibody was an anti-mouse IgG coupled to horseradish peroxidase. Dramatic increases in signal strength (over 100-fold) were obtained as the number of biotins increased to 8. In the case of multiply phosphotyrosinylated probes, where the primary detection was by a mouse anti-phosphotyrosine monoclonal antibody, increases in sensitivity and signal strength were also observed but of more modest magnitude.

The particular advantage of these non-nucleosidic phosphoramidite reagents is that they can be used in place of conventional nucleoside phosphoramidites for the direct incorporation of multiple haptens and other reporter groups into the oligonucleotide chain at any positions required (5'-end, 3'-end or at any internucleotide positions) and there is no need for post-assembly functionalisation chemistry to prepare the probes.

REFERENCES

1) Nelson. P.S., Sherman-Gold, R. and Leon, R. (1989) *Nucleic Acids Res.*, **17**, 7179-7194.

2) Roget, A., Bazin, H. and Teoule, R. (1989) *Nucleic Acids Res.*, **17**, 7643-7644.

3) Misiura, K., Durrant, I., Evans, M.R. and Gait, M.J. (1990)*Nucleic Acids Res.*, **18**, in press.

4) Whitehead, T.P., Thorpe, G.H.G., Carter, T.J.N., Groucutt, C. and Kricka, L.J. (1983) *Nature*, **305**, 158-159.