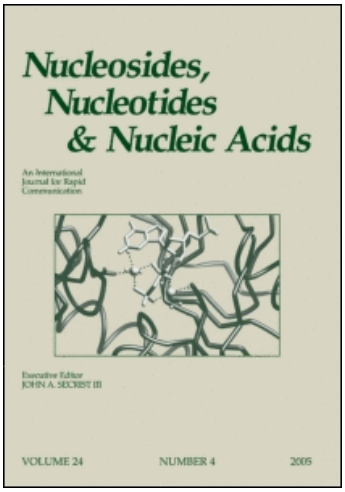


Nucleosides, Nucleotides and Nucleic Acids

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

Nitroindoles as Universal Bases

D. Loakes[a]; F. Hill[a]; S. Linde[b]; D. M. Brown[a]
[a] Laboratory of Molecular Biology, Medical Research Council, Cambridge [b] Department of Haematology, University of Cambridge, Cambridge, United Kingdom

To cite this Article Loakes, D. , Hill, F. , Linde, S. and Brown, D. M.(1995) 'Nitroindoles as Universal Bases', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 1001 — 1003
To link to this Article: DOI: 10.1080/15257779508012520
URL: http://dx.doi.org/10.1080/15257779508012520

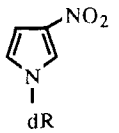
NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 1001-1003 (1995)

NITROINDOLES AS UNIVERSAL BASES.

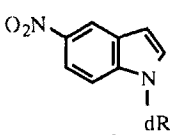
D. Loakes, F. Hill, S. Linde† and D.M. Brown*. Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH. †Department of Haematology, University of Cambridge, Hills Road, Cambridge, United Kingdom.

ABSTRACT. Nitroindoles behave as non-discriminatory bases when incorporated into oligodeoxynucleotides, without causing significant destabilisation of the duplex, and exhibit higher melting temperatures than 3-nitropyrrole. When 5-nitroindole and 3-nitropyrrole are incorporated into oligomers for use as primers for sequencing and the polymerase chain reaction, both give products but are not as effective as hypoxanthine.

There have been a number of attempts to prepare modified bases which behave in a non-discriminatory manner towards the natural bases for oligonucleotide primers and probes. Following an earlier report[1] we began to investigate the nucleoside, **1**, of 3-nitropyrrole, a possible universal base. This work has subsequently been detailed[2], but in the course of our studies we found an alternative to **1**, 1-(2-deoxy-β-D-ribofuranosyl)-5-nitroindole, **2**. This nucleoside was readily prepared in a one step reaction from the indole, and following normal procedures was incorporated into oligonucleotides.



1



2

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I  5' ACTTGGCCACCATTTTG
II 3' TGAACCGGTGGTAAAAC
```

In duplex stability studies with 17-mers (duplexes I and II), 5-nitroindole and 3-nitropyrrole were incorporated opposite each of the natural bases, replacing the central T in duplex II. The melting temperature (Tm) range was found to be 3°C in both cases demonstrating that they each behave ambivalently towards the natural bases. However, 5-nitroindole gave higher Tms than 3-nitropyrrole which is therefore more destabilising. Substitution in the centre of the duplex had a more detrimental affect than was observed at the ends for both modifications. When multiple substitutions were made at the 5'- end with 5-nitroindole, the Tm was maintained to within 7°C compared to the unmodified duplex. However, when similar substitutions were made using 3-nitropyrrole there was a significant

GGT GGA TTC GGC TCA ACT GGA	Template 1
GGT GGC TTT GGA TCG ACT GGA	Template 2
GGA GGA TTT GGA TCC ACG GGG	Template 3
CCN CCN AAN CCN AGN TGN CC	Primer

Figure 1

destabilisation with increasing substitutions. The incorporation of six 5-nitroindoles gave a melting temperature of 50°C as compared to 45°C for three 3-nitropyrroles and 43°C for three mismatches[3]. 4- and 6-Nitroindole behaved qualitatively the same as 5-nitroindole, but were slightly more destabilising. The order of stability was found to be 5-nitroindole > 4-nitroindole > 6-nitroindole > 3-nitropyrrole.

5-Nitroindole and 3-nitropyrrole were incorporated in the primer sequence (Figure 1) using 1, 2 and 6 substitutions (N = 5-nitroindole, 3-nitropyrrole or hypoxanthine) and these were used for Sanger dideoxy sequencing and in the polymerase chain reaction against a sequenced fragment of *Caenorhabditis elegans* genomic DNA cloned into the phagemid vector pBluescriptII SK-.

The ability of each oligomer to prime DNA synthesis in sequencing was determined using Sequenase II, a modified T7 DNA polymerase. It was found that with the incorporation of one or two 5-nitroindole or 3-nitropyrrole residues into the primers that sequencing ladders could be obtained, although with 5-nitroindole these were weak. However, six substitutions in each degenerate position failed to give a sequence in contrast to the results reported by Nichols *et al.*[2] When six hypoxanthines were incorporated then a sequencing ladder was obtained.

When the primers were used in PCR they all gave products of the right length, with the 5-nitroindole working marginally better than the 3-nitropyrrole, in particular when there were two substitutions. When six substitutions were made, however, there was very little product. These results are in contrast to the primer containing six inosines which gave a good yield of product, markedly better than for both 5-nitroindole and 3-nitropyrrole. This again is in disagreement with the results reported by Nichols *et al* [2].

It must be concluded therefore, that whilst all three of the nitroindoles described as well as 3-nitropyrrole behave as universal bases in duplex melting experiments, they are less efficient than inosine in sequencing and PCR.

ACKNOWLEDGEMENTS: D.L., F.H. and D.M.B. thank the MRC for financial support and S.L. thanks Dr. A. Karpas for support.

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