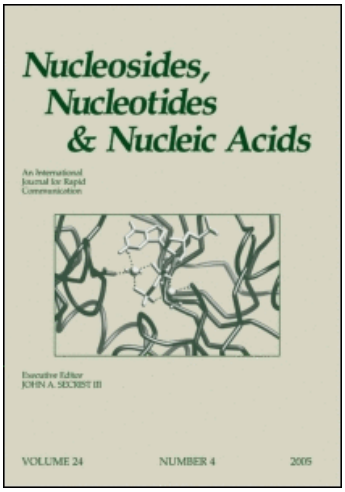

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Synthesis and Polymerase Incorporation Properties of a Tricyclic Pyrrolopyrimidine Related to N⁶-Hydroxy-2'-deoxyadenosine

F. Hill[a]; D. Loakes[a]; C. L. Smith[b]; D. M. Williams[c]; D. M. Brown[a]
[a] Laboratory of Molecular Biology, Medical Research Council, Cambridge, UK [b] Amersham Laboratories, Nycomed Amersham plc, Buckinghamshire, UK [c] Department of Chemistry, University of Sheffield, Sheffield, UK

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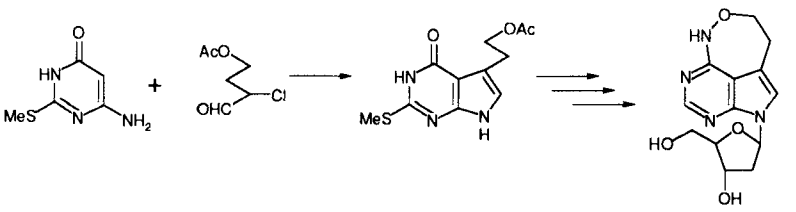
SYNTHESIS AND POLYMERASE INCORPORATION PROPERTIES OF A TRICYCLIC PYRROLOPYRIMIDINE RELATED TO N^6-HYDROXY-2'-DEOXYADENOSINE

F. Hill,[1] D. Loakes,[1] C.L.Smith,[2] D.M. Williams[3] and D.M. Brown.[1]*
[1]Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK.
[2]Nycomed Amersham plc, Amersham Laboratories, White Lion Road, Amersham, Buckinghamshire, HP7 9LL, UK
[3]Department of Chemistry, University of Sheffield, Brook Hill, Sheffield, S3 7HF, UK.

ABSTRACT. The tricyclic nucleotide dLTP has been synthesised. It is incorporated into DNA using a variety of polymerases opposite both template T and dC *in vitro* and *in vivo*.

The tricyclic pyrrolopyrimidine deoxynucleoside, **1**, (denoted as dL) has been prepared in 11 steps starting from the reaction of 2-chloro-4-acetoxybutan-1-al with 2-methylsulfanyl-6-aminopyrimid-4-one, shown in Figure 1.[1] This was then converted to it's 5'-triphosphate to study its incorporation into DNA using a variety of DNA polymerases.

Figure 1



1 (dL)

Incorporation of the pyrrolopyrimidine 5'-triphosphate dLTP by polymerases

The incorporation of dLTP (at 50μM) into DNA, and its specificity were examined in primer extension assays using *Taq* polymerase, DNA polymerase I and its derivatives Klenow fragment and exonuclease-free Klenow fragment, and T4 DNA polymerase. All polymerases except T4 DNA polymerase can incorporate dLTP in place of dGTP or dATP. T4 DNA polymerase may incorporate and rapidly edit dLMP; this possibility is being examined.

Sequencing: dATP and dGTP were replaced by dLTP in radiolabelled terminator cycle sequencing reactions. The concentration of normal dNTPs is 7.5μM. Use of 40μM dLTP for 7.5μM dATP produced a sequence only slightly shorter than that obtained with the 4 normal dNTP's, but longer than that obtained with dITP. Replacing dGTP with dLTP did not give any extension products under the conditions tested.

Terminal deoxynucleotidyl transferase (TdT): Polymerisation of dLTP by the enzyme TdT was examined by tailing a 23-mer oligonucleotide and compared to tailing using dGTP and dATP at the same concentration. The tails with dATP were shorter than those produced with dLTP which extended to greater than 100 bases. Tails produced with dGTP were shorter, as expected, than the dATP tails.

Mutagenesis *in vivo* and *in vitro*: *In vivo* mutagenesis by dL was determined using the six Cupples and Miller strains of *E. coli* as described.[2] No mutations were induced by dL. However, when the strains expressed a broad specificity deoxyribonucleoside kinase, derived from *Drosophila melanogaster* (FH, unpublished), dL proved to be a very potent transition mutagen in the two strains, CC102 and CC106, which revert to lac^+ by the mutations GC→AT and AT→GC respectively.

To determine the spectrum of mutations produced by dLTP *in vitro*, PCR reactions were supplemented with dLTP (used at 100μM; normal dNTPs were at 50μM) and the amplified DNA cloned and sequenced. The supF gene of *E. coli* was used as a template for amplification to allow comparison with the results obtained using dZTP and dKTP.[2] Only transition mutations were obtained, as expected. The most notable finding was that, in marked contrast to dKTP and dZTP, the number of A:T to G:C mutations (16) was very similar to the number of G:C to A:T mutations (14).

Conclusion: dLTP is a very good polymerase substrate. The nmr spectra of the nucleoside strongly suggests that one tautomeric form, A-like, is favoured, as was found with N^6-methoxydA.[2] As a polymerase substrate, dLTP can behave as either dATP or dGTP, as was also found with N^6-methoxydA.[2] However, dLTP gives an even balance of A→G and G→A transition mutations, unlike N^6-methoxydA (ratio of A→G versus G→A; 10:1).

REFERENCES

1. Williams, D. M., Loakes, D. and Brown, D. M., (1998), J. Chem. Soc. Perkin 1, in press.
2. Hill, F., Williams, D. M., Loakes, D. and Brown, D. M., (1998), Nucl. Acids Res., **26**, 1144-1149.