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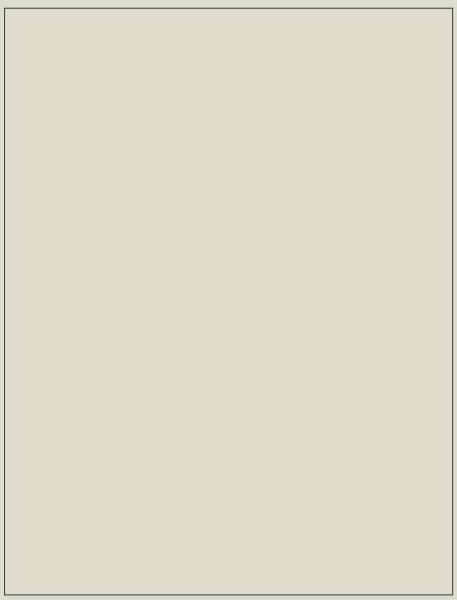




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ARTICLE

# Azide and *trans*-Cyclooctene dUTPs: Incorporation into DNA Probes and Fluorescent Click-Labelling

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5-Azidomethyl dUTP and two 5-*trans*-cyclooctene dUTPs with different linkers between the TCO and the uracil base have been incorporated into DNA by primer extension, reverse-transcription and PCR amplification. For azidomethyl dUTP the PCR reaction was successful even when the modified dUTP was not supplemented with dTTP. In one case 335 azidomethyl dU residues were incorporated into the 523 base pair amplicon using this methodology. Although 5-azidomethyl dUTP was found to be a better substrate for DNA polymerases than the *trans*-cyclooctene dUTPs, the inverse electron demand Diels–Alder reaction between cyclooctene DNA and a tetrazine Cy3-dye was more efficient than the strain-promoted reaction between azide DNA and a bicyclo [6.1.0] non-4-yne Cy3 dye.

## Introduction

Nascent DNA synthesis can be monitored in proliferating cells by fluorescence. Cells are incubated with the thymidine analogue 5-ethynyl-2′-deoxyuridine which is converted to its triphosphate by cellular enzymes and incorporated into genomic DNA by endogenous polymerases. Subsequent cell-fixation and derivatisation of the alkyne-modified DNA with a fluorescent azide (e.g. TAMRA azide) *via* the CuAAC reaction[1, 2] produces labelled DNA (Figure 1.A) which can be visualised by fluorescent cell imaging.[3] The same approach has been adapted to detect RNA synthesis in cells by incorporating the ribonucleoside 5-ethynyluridine into newly transcribed RNA.[4] The methodology is compatible with fixed cells, but the toxicity of copper precludes its use on live cells. Recently it has been shown that the need for toxic metal ion catalysis is negated if an azide-modified nucleoside is incorporated into genomic DNA and a cyclooctyne derivative of the dye[5] (such as bicyclo [6.1.0] non-4-yne (BCN))[6], is used in the fluorescent labelling step (Figure 1.B).[7] Relief of ring strain by a change of hybridisation from sp to $sp^2$ drives a fast chemical reaction between the cyclooctyne attached to the dye and the azide-modified DNA. This reaction, known as the strain-promoted alkyne-azide cycloaddition (SPAAC) reaction,[7, 8] has also been used to label synthetic oligonucleotides.[9] Unlike bulky dye-labelled nucleosides, ethynyl dU and azidomethyl dU are sufficiently similar to their natural congener (thymidine) to be converted to their triphosphates by endogenous enzymes within cells. This intracellular conversion of nucleoside to dNTP is essential; unlike free nucleosides, dNTPs cannot be used in cell-labelling experiments as they do not pass through the cell membrane.

In addition to cell imaging, there are many applications that require labelled DNA, for which click chemistry is particularly useful.[10] A popular approach is to incorporate functional groups such as alkynes into the DNA during PCR amplification, primer extension or nick translation *via* a modified dNTP, and to subsequently label the modified DNA with an azide derivative of a reporter group such as a fluorescent dye.[11-14] Similarly, azides can be incorporated into DNA or RNA for fluorophore attachment *via* CuAAC, SPAAC or Staudinger reactions.[15-18] Such two-step labelling processes are necessary because direct enzyme-mediated incorporation of fluorophore-labelled dNTPs during PCR is inefficient due to the bulky nature of fluorescent dyes. In contrast, dNTPs that are modified by addition of sterically undemanding groups such as terminal alkynes are incorporated much more efficiently.

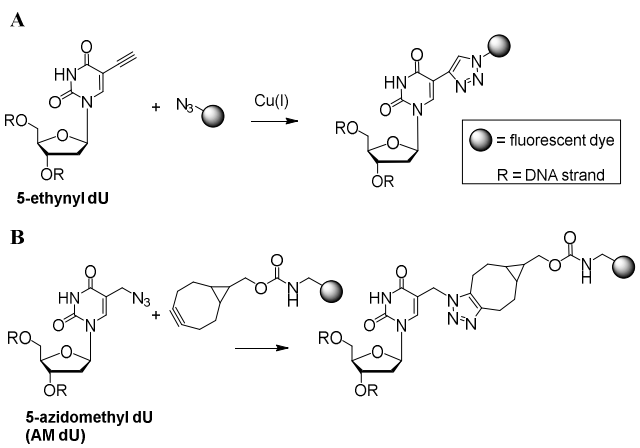


Figure 1. Fluorescent DNA labelling in cells: A). 5-Ethynyl dU and a fluorescent azide (CuAAC); B). 5-Azidomethyl dU and a BCN-labelled fluorophore (SPAAC).

Crystal structures of the thermostable DNA polymerase KlenTaq with alkyne-modified substrate dNTPs in its active site illustrate the plasticity of the enzyme and the presence of flexible regions of the dNTP binding pocket. Due to these