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Termination of DNA synthesis by novel 3'-modifieddeoxyribonucleoside 5'-triphosphates

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Abstract

Eight 3'-modified-dNTPs were synthesized and tested in two different DNA template assays for incorporation activity. From this enzymatic screen, two 3'-O-methyl dNTPs were shown to terminate DNA syntheses mediated by a number of polymerases and may be used as alternative terminators in Sanger sequencing. 3-O-(2-Nitrobenzyl)-dATP is a UV sensitive nucleotide and was shown to be incorporated by several thermostable DNA polymerases. Base specific termination and efficient photolytic removal of the 3'-protecting group was demonstrated. Following deprotection, DNA synthesis was reinitiated by the incorporation of natural nucleotides into DNA. The identification of this labile terminator and the demonstration of a one cycle stopstart DNA synthesis are initial steps in the development of a novel sequencing strategy.

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