Abstract

This work is focused on the synthesis of the modified 2'-deoxyribonucleoside triphosphates, their incorporation into DNA and use in chemical biology applications. The synthetic routes to the double-headed nucleosides and nucleotide triphosphates in which the two nucleobases were connected via ethynyl or propargyl linker has been developed. (Cytosin-5-yl)ethynyl, 3-(cytosin-1-yl)prop-1-yn-1-yl and 3-(5-fluorocytosin-1-yl)prop-1-yn-1-yl derivatives of pyrimidine and 7-deazaadenine 2'-deoxyribonucleosides and nucleoside triphosphates were prepared by aqueous palladium-catalyzed cross-coupling reactions. The double-headed modified nucleoside triphosphates were good substrates for DNA polymerases suitable for primer extension and PCR construction of DNA bearing linked cytosine or 5-fluorocytosine in the major groove mimicking the flliped-out nucleotide. The assay for the testing of the inhibition of DNA methyltransferases was developed. Next, the transient protection of DNA against cleavage by restriction endonucleases (REs) using (trialkylsilyl)ethynyl modified DNA was developed. A series of 7-(trialkylsilyl)ethynyl-7-deaza-2'-deoxyadenosine triphosphates was prepared and they were shown to be incorporated into DNA by primer extension and/or PCR using KOD XL polymerase. The deprotection conditions of the trialkylsilyl protecting groups were optimized on model nucleoside monophospahtes. The ability to protect the DNA against cleavage by REs by using (trialkylsilyl)ethynyl modifications was tested. It was found that the (triethylsilyl)ethynyl-protected DNA resists the cleavage by RE, but after it is treated with NH_3, the resulting deprotected ethynyl-modified DNA is fully cleavable by the REs. This 7-(triethylsilyl)ethynyl-7-deaza-2'-deoxyadenosine triphosphate was also used in a PCR-based synthesis of a gene internally protected against cleavage by restriction endonucleases. The unmodified flanking regions were cleaved for cloning into a plasmid which was replicated by *E. coli*, and used for protein production. Finally, a series of 7-substituted 7-deazaadenine and 5-substituted cytosine 2′-deoxyribonucleoside triphosphates were tested for their competitive incorporations (in the presence of their natural counterparts) into DNA by several DNA polymerases by using analysis based on cleavage by restriction endonucleases. 7-Aryl-7-deaza-2'-deoxyadenosine triphosphates were shown to be more efficient substrates than dATP because of their higher affinity for the active site of the enzyme, as proved by kinetic measurements and calculations.