Summary

In the first part of the thesis, a series of six modified 2'-deoxyadenosine triphosphates, bearing small functional groups (chloro, amino, methyl, vinyl, ethynyl and phenyl) at position 2 of adenine, was designed and synthesised. They were then tested as substrates for DNA polymerases in enzymatic synthesis of minor-groove modified DNA. The 2-phenyl modified dATP was the only triphosphate unable to be incorporated, meaning that the phenyl group is already too big for minor-groove incorporations. All of the other tested nucleotides were good substrates for tested DNA polymerases [KOD XL, Vent(exo-) and Bst LF] affording minor-groove modified DNA bearing one or four modifications. The vinyl- and ethynyl-modified DNAs were then used for post-synthetic modification of DNA minor groove with fluorescent labels utilising click reactions. Ethynyl group reacted in copper-catalysed alkyne-azide cycloaddition (CuAAC), whereas the vinyl group participated in thiol-ene reaction. This procedure allowed for the attachment of big functional groups otherwise unable to be installed into the DNA minor groove using direct enzymatic incorporation.

The second part of the thesis was devoted to the study of 2-alkylamino-2'-deoxyadenosine triphosphates and their use in enzymatic synthesis of base-modified ONs and DNA. These N^2-alkyl diaminopurine nucleotides were synthesised and tested as substrates for DNA polymerases. The methylamino modified derivative was the only nucleotide which KOD XL DNA polymerase efficiently incorporated into DNA. All of the other nucleotides were poor for all tested DNA polymerases in those experiments. Therminator DNA polymerase was however able to extend the primer by incorporation of one modified adenine bases, when no other 2'-deoxynucleoside triphosphates (dNTPs) were present in the reaction mixture. Optimisation of reaction conditions for the experiment with template encoding for two consecutive adenine bases allowed for incorporation of either one or two modified triphosphates. When an excess of natural dNTPs was added to the reaction mixtures, PEX using these nucleotides resulted in site-specific minor-groove modified DNA. The allyl- and propargylamino-modified DNAs were then used for post-synthetic transformations of DNA minor-groove using click reactions with fluorescent labels. Ultimately this approach was used for the construction of DNA FRET probes, useful for the monitoring of DNA duplex denaturation/reannealing process or for the detection of specific ON sequences.

Finally, we also extended the portfolio of modified nucleotides useful for construction of minor-groove modified DNA to dGTP analogues. We prepared a series of five 2-substituted 2'-deoxyinosine triphosphate derivatives, bearing small substituents (chloro, methyl, vinyl, ethynyl and phenyl), which acted as surrogates of guanine nucleobase and base-paired with cytosine. Therminator DNA polymerase was able to produce full-length products with the 2-methyl and 2-vinyl modified nucleotides, whereas the other derivatives were not incorporated by any of the tested DNA polymerases. The prepared vinyl-modified DNA participated in thiol-ene reactions with thiols and also with a cysteine-containing minor-groove binding peptide providing a successful product of crosslinking thanks to the proximity effect of the functional groups.

All of the prepared 2-substituted purine nucleotides were ultimately used to study the influence of minor-groove modifications on cleavage of dsDNA by tape II restriction endonucleases. No general trend for the effect of these modifications was observed. Both enzymes cleaving the minor-groove modified DNA and enzymes whose activity was inhibited by these modifications were identified.