2-SUBSTITUTED dATP DERIVATIVES AS BUILDING BLOCKS FOR ENZYMATIC SYNTHESIS OF DNA MODIFIED IN THE MINOR GROOVE

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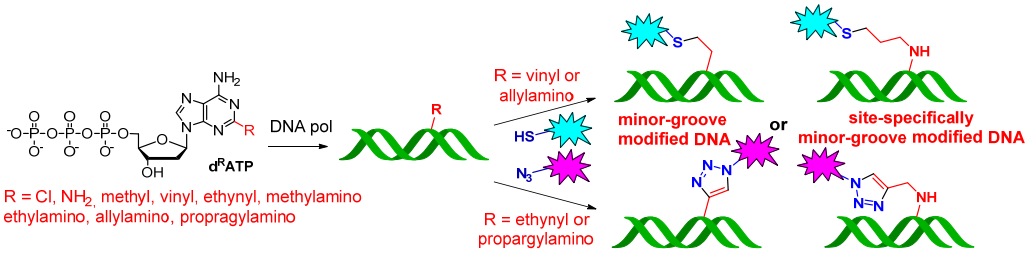
Enzymatic preparation of modified DNA, interesting due to potential applications in chemical biology or bioanalysis, requires the use of DNA polymerases and (base-)modified nucleoside triphosphates (dNTPs). The substrate activity of these enzymes is retained when the nucleobases are modified at a position that allows for the modification to be located inside the major groove of DNA.[1] However, enzymatic production of minor-groove modified DNA was still unexplored and worth pursuing.

Series of 2-modified-2'-deoxyadenosine triphosphates modified either by small substituents (Cl, amino, methyl, vinyl, ethynyl and phenyl) or by alkylamino groups (methyl-, ethyl-, allyl- and propargylamino) were synthesized.[2] These prepared d^RATPs were then tested as substrated for DNA polymerases and were efficiently used for synthesis of fully or site-specifically minor-groove modified DNA. DNA minor groove was further functionalized by click reactions with fluorescent labels. This approach was ultimately used for the construction of FRET probes for the detection of oligonucleotides.



Scheme 1: Polymerase synthesis of DNA modified in the minor groove and post-synthetic modification by fluorescent labels utilizing click chemistry

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