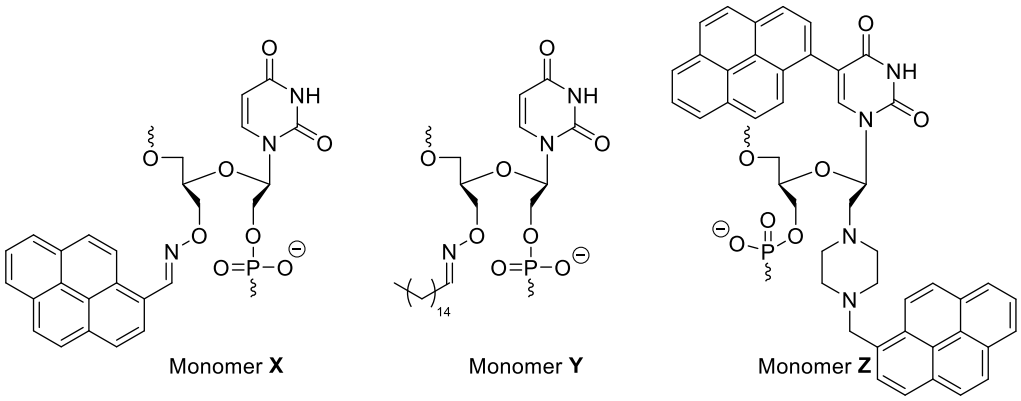
Abstract

The growing interest for use of oligonucleotides (ONs) as therapeutics calls for development of new functionalized nucleotide monomers and methods for easy functionalization of ONs. This thesis concerns the modification of ONs and is divided into two parts: 1) synthesis of novel Unlocked Nucleic Acid (UNA) building blocks (chapter 2), 2) postsynthetic modification of ONs by conjugation chemistry (chapter 3 and 4).

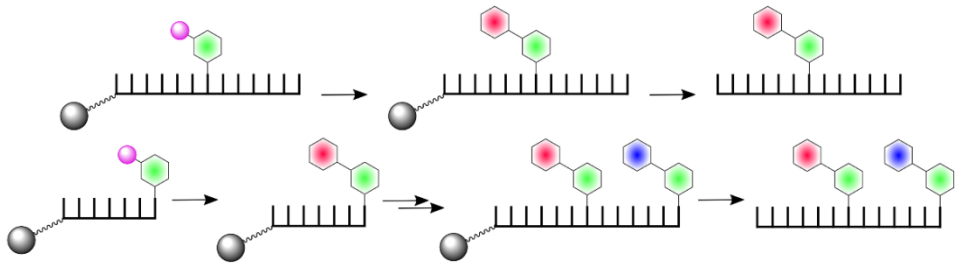
UNA was functionalized in the 3'-position with pyrenyl and palmityl via coupling to 3'-*O*-amino-UNA creating an oxime linker. Thermal denaturation experiments showed that introduction of the pyrene-modified monomer **X** led to an unprecedented thermal stabilization of DNA:DNA and 2'-*O*-Me-RNA:DNA duplexes but with a small thermal destabilization of DNA:RNA and 2'-*O*-Me-RNA:RNA duplexes. The palmityl-modified monomer **Y** introduced a thermal destabilization to the aforementioned duplexes equivalent to unmodified UNA. Mismatch discrimination of the modified duplexes could be modeled depending on the number of modifications introduced and the position to the mismatch. Fluorescence emission spectroscopy of duplexes containing monomer **X** showed formation of an excimer for some single-stranded constructs with multiple modifications which was significantly reduced upon hybridization with a DNA or RNA complement. Moreover, a stabilization of parallel triplexes was observed when monomer **X** was incorporated into the triplex-forming strand. Moreover, monomer **Y** modified antisense ONs could lower the expression of apoB mRNA both gymnotic and with lipofectamine showing a cooperative effect of the alkyl chain and the UNA scaffold.



A bis-pyrene-modified UNA monomer **Z** was also synthesized which proved to destabilize modified DNA:DNA and DNA:RNA duplexes. An excimer was observed for single-stranded DNAs with double modifications and the excimer intensity was significantly reduced upon

hybridization with a complementary strand. Moreover, differences in the fluorescence output at 380 and 450 nm could distinguish between single-stranded ONs, duplexes with a DNA/RNA complement or duplexes with a DNA/RNA complement where monomer **Z** was placed as a single-nucleotide bulge. This makes the monomer suitable for a probe detecting single point deletions.

Two new methods were developed for the on-column functionalization of DNA containing 5-Iodo-2'-deoxyuridine (5IdU) by use of Suzuki cross-coupling chemistry. Single modified 9mer DNAs were subjected to Suzuki couplings with three different boronic acids showing a 100% conversion into the respective cross-coupling products. Efficient couplings were carried out with full-length ONs and immediately after introduction of 5IdU with superior yields for the latter. Coupling reactions with triple modified 9mer DNAs were also successful and the yield for the fully modified product was increased by extension of the reaction time and employing double couplings. Again, higher yields were observed for coupling reactions immediately after incorporation of 5IdU. Moreover, Suzuki couplings were used to introduce three different modifications to the same DNA ON.



An 8mer and a 12mer DNA ON was conjugated to the AT-hook peptide using Cu-catalyzed click chemistry. The AT-hook peptide is a known minor groove binder that binds AT-rich regions of DNA duplexes. POC:DNA duplexes showed increased thermal stability compared to unmodified DNA duplex and also to a mixture of the DNA duplex and unbound AT-hook peptide. This showed an increased binding affinity of the protein by covalent attachment to DNA. Introduction of mutations in the AATT-binding region of the duplex impeded duplex formation altogether showing distinctive sequence specificity for the peptide. Mutations in the binding region of the peptide did not give a significant increase in thermal stability for POC:DNA duplexes but did also not impede duplex formation showing no interactions between the peptide and ON. Covalent attachment of the AT-hook peptide to DNA leads to an increase in target affinity which can be utilized in therapeutic applications.