ABSTRACT

Conformationally restricted and chemically modified nucleotides are imperative in oligonucleotides drugs because of their ability to pre-organize the sugar puckering and therein to gain favorable affinity and potentially increase bioavailability and safety. The exploration of novel conformationally restricted nucleotides might pave the way for novel indications of oligonucleotide-based drugs and is therefore a key driving force for innovation within nucleic acid based biotechnology. In this dissertation six novel constrained nucleotide (or alike) monomers that were incorporated into oligonucleotides (or oligomers) are presented. The experimental work of this thesis is divided into three chapters (CHAPTER 4, 5 and 6).

CHAPTER 4 is further divided into three parts. The first part presents synthesis of phosphorodiamidite synthon of 2′-amino-LNA-T **4.2** as a constrained morpholine(o) monomer and a method to incorporate it into DNA oligonucleotides with an adjacent phosphoramidate or dimethylamino phosphordiamidate (through oxidative substitution of a boranophosphonate oligonucleotide intermediate) linkage as monomers **X** and **Y**, respectively. The latter backbone modification showing, to lesser extent than the former, a less thermal destabilizing effect because of a decreased electrostatic repulsive effect.

The second part presents the novel synthesis of a 2′*N*-3′*O*-cyclophosphoramidate of 2′-amino-LNA-T **4.10** via the method of oxidative substitution of a boranophosphonate intermediate. The product was however sensitive to hydrolysis and gave the 3′-*O*-phosphate derivative progressively.

The third part presents the synthesis and introduction of constrained PNA monomer utilizing a morpholine heterocycle of **4.16** into PNA oligomers as monomer **M**. The affinity towards complementary DNA and RNA were however completely decimated with incorporation of this constrained modification even with glycine spacers.

CHAPTER 5 presents the synthesis and introduction into DNA oligonucleotides of two novel constrained 2′-methylribonucleotides (2′-*C*-Me-LNA-U **5.10** and 2′-*C*-Me-ONA-U **5.13**) as monomers **W** and **Q**, respectively. The LNA derivative showed a thermal destabilizing effect and an X-ray structure of the intermediate **5.6** in the synthesis gave structural insight to this feature. The sugar puckering of the LNA derivative **5.6** was indeed in a 3′-*endo* (*North*-type) conformation, however a sterical clash was evident between the 2′-Me substituent and the nucleobase which likely impeded the nucleobases' ability to form adequate Watson-Crick base pairing. The ONA derivative showed however when incorporated thrice to be selective against its complementary RNA over DNA by a gap of 10°C. The 2′-*C*-Me-ONA derivatives might be a suitable candidates for antisense activity because of this selectivity.

CHAPTER 6 presents the synthesis and incorporation of a novel phosphoramidite of 2′-fluoro-3′-*C*-hydroxymethyl-2′-deoxy-ANA **6.9** into DNA oligonucleotide as monomer **Z** to utilize the Gauche effect by the 2′-F substitution to increase the 2′-*endo* (*South*-type) conformation compared to a previously described non-fluorinated analogue. A destabilizing effect was however observed with one incorporation and questionable stability of the oligonucleotide with three incorporation.