CHAPTER IV

CONCLUSION

PNA oligomers carrying β-amino acid have been successfully prepared. These PNA oligomers consist of a monomer and a spacer. The PNA monomers were derived from proline by attachment of nucleobases at the C-4 position. The thymine monomers were prepared in all 4 diastereomers ("*cis*-D", "*trans*-D", "*cis*-L" and "*trans*-L"). For others nucleobase (adenine, cytosine and guanine) only the "*cis*-D" configuration was prepared. The spacer part derived from β-amino acids, which may be categorized into three classes: acyclic, cyclic containing one stereogenic center and cyclic containing two stereogenic centers. All PNA monomers, including β-amino acid spacers were prepared with *N*-Fmoc protection and activated at the *C*-terminal with Pfp ester for use in solid-phase peptide synthesis.

Oligomerization of PNA was accomplished by Fmoc solid-phase peptide synthesis to give a number of PNA with various sequences. These PNAs were purified by reverse phase HPLC and characterized by MALDI-TOF mass spectrometry. Binding of the PNA oligomers with DNA were investigated by UV-titration and T_m measurement. These revealed that the PNA systems containing *cis*-D proline and D-aminopyrrolidine carboxylic acid (D-APC) and (1*S*,2*S*)-2-aminocyclopentane carboxylic acid [(1*S*,2*S*)-ACPC] spacers can form 1:1 hybrids with complementary DNA. PNA with other spacers or different configurations of the proline ring did not show any binding indicating a stringent requirement of structural organization. Further binding properties of *cis*-D/D-APC PNA were then examined by T_m, gel electrophoresis, CD spectroscopy and fluorescence experiments. Higher T_m were observed with longer PNA·DNA hybrids. T_m studies of *cis*-D/D-APC PNA (T_4XT_4) with DNA (dA_4YA_4) showed that the complementary T_9 and A_9 gave the highest T_m around 68.8 °C compared with others perfect match complementary (60.0 °C for X = A, Y = T, 46.7 °C for X = C, Y = G and 56.4 °C for X = G, Y = C). A single mismatch at the central positions in the DNA strands caused a drop in T_m of 13-34 °C, indicating a high Watson-Crick specificity in recognition of DNA by these

PNA. Ionic strength and pH effect had relatively little effect on the T_m of PNA·DNA, which is similar to Nielsen's PNA·DNA hybrid but opposite to DNA·DNA hybrid.

The direction of binding was also explored by T_m (T_m <30 °C for parallel form, T_m = 65 °C for antiparallel form) and fluorescence experiment. The FRET phenomenon was observed when the PNA bound with antiparallel DNA probe. From these experiments, it can be concluded that the *cis*-D/D-APC PNA system strongly favors forming antiparallel hybrid with DNA. This has also been confirmed by molecular dynamics simulations.