CHAPTER IV

CONCLUSION

The actived PNA monomers containing all four nucleobases have been successfully prepared for solid phase synthesis from proline derivatives with the nucleobase (thymine, adenine, guanine and cytosine) attached at C-4 position in *cis*-D stereochemistry. The Fmoc-protected monomer was chosen due to its many advantages over Boc-protected monomers. The free acids were converted into Pfp esters by reacting with PfpOH/EDC.HCl to give the desired *cis*-D pyrrolidinyl monomers. For the synthesis of the *ss*ACPC spacer, ethyl 2-cyclopentanone carboxylate and (*S*)-(-)-α-methylbenzylamine were used as starting materials. After a removal of the chiral auxiliary group by hydrogenation, followed by hydrolysis and protection of the amino group with FmocOSu, an Fmoc-free acid was obtained. Treatment with PfPOH/ EDC.HCl afforded the N-protected, C-activated monomer as described previously for PNA monomers. The structure of pyrrolidine monomers and ssACPC spacer were characterized by NMR and mass analysis

Oligomerizations were carried out using Fmoc SPPS coupling strategy. In case of sequence containing base T, A and C, the average coupling yields were approximately over 95 % per step under the standard protocol. However, for the sequence containing base G, the efficiency for the next coupling step of nucleobase monomer always dropped to 80 %. To improve the coupling efficiency for this step, varying the coupling reagent and additive was investigated. However, the efficiency still drops to ~80 % regardless of the coupling condition. Thus, a purification step based on the use of a hydrophobic capping reagent such as long-chain carboxylic acid chloride or anhydride has been developed. The retention time of incomplete sequences containing the hydrophobic tag are longer than the complete PNA (which contains only an acetyl cap at the N-terminus). In the coupling step, Pfp/HOAt method was original used; DIEA was also added as additive to improve the reaction rate. In the presence of DIEA, over 90 % coupling efficiency was obtained in coupling time only 30 min which much decreased from original coupling time of 2 h.

The hybridization properties of mixed-base *ss*ACPC PNA was investigated by UV melting analysis and CD spectroscopy. T_m studies of homothymine PNA (T_n) with complementary DNA ($d(A_n)$) formed stable complex with high affinity (T_m = 54 °C for n = 7, T_m = 77 °C for n = 9 and T_m = >85 °C for n = 10). The effect of ionic strength and pH effect had relatively little effect on T_m of PNA·DNA, which is similar to Nielsen's PNA·DNA hybrid but opposite to DNA·DNA hybrid. The UV titration between *cis*-D homothymine (**12f**) and poly(dA) shown a 1:1 stoichiometry of T:A, indicating the formation of a double helical complex.

To study the base pairing specificity in polypyrimidine sequences (**12f-12i**) and polypurine sequences (**12j-12m**) containing, all possible pairs of nucleobase interaction was investigated. The hybrid formed from complementary PNA and DNA possessed the highest T_m value whereas the duplex containing a single mismatch had a significantly decreased T_m. The result confirmed that the Watson-Crick base-pairing specificity in PNA·DNA hybrids is followed in both pyrimidine- and purine-rich environments.

For mixed-base sequences, the hybridization between PNA and DNA can be concluded as follow. The single mismatches in the middle position have greater effects on the hybrid stability more than other positions. For the effect of mismatch base pairing, all possible single mismatches caused a drop in T_m of 10-18 °C in 15-mer. Deletion and insertion in the DNA strand also resulted in a the drop of T_m around 12-18 °C. Furthermore, the hybridization between PNA·DNA, PNA·RNA and PNA·PNA with identical base sequences showed that these PNA system can bind selectively with DNA over RNA and over self pairing. This property has not been previously reported in any other PNA systems including the original *aeg*PNA of Nielsen.