CHAPTER IV

CONCLUSION

Three novel chiral and conformationally constraint peptide nucleic acids (cPNA) carrying hydrophilic side chain have been synthesized. The synthetic process required an intermediate, Boc-D-Pro(*cis*-4-T^{Bz})-ODpm **(7)** which was synthesized from *trans*-4-hydroxy-L-proline **(1a)**. Epimerization the *trans*-L-epimer into *cis*-D-epimer **(1b)** were made by heating the amino acid in acetic anhydride followed by hydrolysis. Fractional crystallization from methanol instead of ethanol provide a better yield than previously reported. Incorporation of thymine into C-4 position of proline with retaining the *cis*-D configuration have been made by double Mitsunobu reaction of fully protected *cis*-D-proline *via* S_N^2 displacement. Crystallization of the intermediate product from methanol gave a better yield **(7)** and could avoid chromatographic purification.

Both L- and D-diastereomers of serylproline cPNA monomer [**(12)** and **(13)**] was synthesized by coupling of the intermediate **(7)** after removal of the N-terminus Boc protecting group with each serine isomer in the presence of DCC as coupling agent, HOBt as auxiliary nucleophile and DIEA as base. Selective deprotection of the C-terminal protecting group followed by activation with pentafluorophenol/DCC gave the desired serylproline diastereomeric monomers. Oligomerization were carried out employing Fmoc/O^tBu fragment coupling strategy. The two cPNAs were then purified by reverse phase HPLC and characterized by MALDI-TOF mass spectrometry. The percentage yield were calculated from UV-absorbance of dibenzofulvene-piperidine adduct which gave 40.0 % for L-series **(25)** and 44.6 % for D-series **(26)** respectively.

Deoxyglycylproline monomer **(23)** was synthesized by reacting the intermediate **(7)** with *N*-nosylaziridine **(18)** *via* S_N^2 displacement. The activated aziridine **(18)** was synthesized from ethanolamine and nosyl chloride followed by ring closure with Mitsunobu reaction. Denosylation of the ring opening adduct **(19)** was achieved by masking the nitrogen of aminoethyl moiety with Boc group followed by treatment with thiophenoxide ion. The resulting denosylated product **(21)** was converted into the corresponding Fmoc derivative **(22)** by deprotection and acylation with FmocCl.

Oligomerization of this monomer **(23)** was achieved by HBTU activation strategy and then purified with reverse phase HPLC. Identification of the products were also performed by MALDI-TOF mass spectrometry. The overall yield **(24)** was 17.3 %, calculated from UV-absorbance of dibenzofulvene-piperidine adduct.

The ability of all synthesized decathymine cPNA, some of which contains protecting group to form complexes with fluorescent labelled decaadenylic acid [F(dA_{10})] were studied by gel electrophoresis. The lack of binding of all cPNA to F(dA_{10}) were observed even at higher than 1:1 stoichiometric proportion of F(dA_{10}):cPNA were used. The result are quite unexpected and it is not clear why addition only a small hydrophilic side chain of serine would make these cPNAs completely unable to binding with their complementary oligonucleotides. Nevertheless, we presevere to explain this negative result which include: i) formation of some intramolecular hydrogen bonding at hydroxylic side chain of serine led to stablization of the single strand cPNA. ii) steric effect of serine side chain towards the oligonucleotide backbone to destabilize the complex.

In case of deoxyglycylproline cPNA, these results appeared to contradict the previously report results.[82] However it should be noted that the configurations on the proline ring in this study and previous study are different. In addition, in previous studies of aminoethylproline cPNA, only melting temperature experiment was performed and it showed inconclusive melting curves and thus more experiments are needed to confirm the binding of such cPNA. These unexpected results certainly need more experiments to fully understand the explanation.

Recommendation

This work should be further extended in order to provide a better understanding to the results obtained above. The author would like to suggest some possible further works as follows:

1) Binding of deoxyglycylproline cPNA to its complementary oligonucleotides should be studied by other techniques such as melting temperature, NMR, CD and enzyme assay.

2) Insertion of our cPNA monomer in the original PNA chain at suitable positions should be attempted since it would not reduce binding affinity too much while increasing the water solubility of PNA.

3) Other amino acid especially having charge side chain should be introduced instead of serine to compare the results with this research.

4) Substitution of the aminoethyl part with other related spacers with different charge and substituents.