CHAPTER V

CONCLUSION

Syntheses of both enantiomers of the Fmoc/Boc-protected and Fmoc/Tfa-protected *trans*-APC spacer were successfully achieved following a literature procedure. The APC spacers were synthesized by Diekmann cyclization reaction between Boc-glycine ethyl ester and ethyl acrylate to give the ethyl 3-oxopyrrolidine-4-carboxylic acid (**3**). The chiral enamines derived were synthesized by diastereoselective reduction between β-ketoester (**3**) and (*S*)-(–)-α-methylbenzylamine or (*R*)-(+)-α-methylbenzylamine as chiral auxiliaries. After removal of the chiral auxiliary group by hydrogenation, followed by hydrolysis and protection of amino group with FmocOSu, the N^3-Fmoc, N^1-Boc protected APC was obtained. Treatment with PfpOH/DIEA afforded the *N*-protected (3*R*,4*S*)-APC and (3*S*,4*R*)-APC isomers of the protected spacers in 6.5% and 4.9% overall yield, respectively. The Boc group of compound **8** was removed by treatment with trifluoroacetic acid (TFA) followed by activated with an excess of PfpOTfa to give the Pfp-activated N^1-Tfa-protected (3*R*,4*S*)-APC spacer **10** in 60% yield. These spacers have been coupled with pyrrolidine monomer and oligomerized into model PNA sequences by Fmoc solid phase peptide synthesis which purified by HPLC and confirmed by MALDI-TOF MS.

The hybridization properties of all *apc/acpc*PNAs were investigated by UV melting analysis and CD spectroscopy. The T_m value of the fully modified homothymine PNA **P2** was 55.5 °C, which was smaller than the unmodified PNA **P1** (75.6 °C). The results showed that the fully modified of new spacer in the pyrrolidinyl PNA system moderately decrease the DNA binding stability. However, the singly modified PNA **P4** carrying (3*R*,4*S*)-APC spacer in middle position of the strand of the (1*S*,2*S*)-ACPC spacer which possess the same absolute configuration as (1*S*,2*S*)-ACPC could bind to its complementary DNA giving a high T_m (71.1 °C) comparable to PNA **P1** (75.6 °C). The effect of ionic strength and pH had a dramatic effect on T_m of the fully modified *apc*PNA **P2** with DNA but had relatively little effect on T_m of the singly modified PNA **P4**. The UV titration between the **P4** and DNA indicated the formation of a double helical complex resemble to *acpc*PNA. On the other hand, the

fully modified PNA **P2** formed a 2:1 PNA:DNA hybrid. The PNA **P3** did not show any equivalent point as expected from the incorrect configuration of the spacer. The T_m of the mixed-base sequences **P6** was only slightly decreased compared to the unmodified *acpc*PNA ($\Delta T_m = -1.4$ °C) upon substitution of one ACPC by one (3*R*,4*S*)-APC unit.

The singly modified *apc*/*acpc*PNA labeled with pyrenecarbonyl and pyrenebutyryl labels (**P7**, **P8**, **P9** and **P10**) have been successfully synthesized on solid support. For the pyrenecarbonyl labeled PNA (**P7** and **P9**), the homothymine nonamer PNA **P7** gave ~3.0-fold at 382 nm increase in fluorescence upon hybridization with complementary DNA, and gave barely noticeable change in the hybridization with a single mismatch DNA target. The mixed-base sequence **P9** with the same pyrenecarbonyl label exhibited fluorescence change (~1.3 fold) under the same circumstance. The pyrenebutyryl labeled PNA (**P8**, **P10**) showed very high differentials of fluorescent intensity over 70 folds at 379 nm of the single-stranded **P8** compared to the weak fluorescence intensity of the hybridization with a single mismatch DNA target. For the mixed base sequence **P10** exhibited the fluorescence intensity was considerably smaller ~4.8 fold compared to the single stranded **P10**.

The results above of new *apc*/*acpc*PNA system modified with a single APC unit in the middle of the strand, suggested that the *trans*-(3*R*,4*S*)-APC spacer was compatible with the original PNA backbone carrying 2-amino-1-cyclopentanecarboxylic acid (ACPC) spacer as shown by their only slightly decreased T_m. The APC residue can be used as a handle for incorporation of fluorescence labels such as pyrene. The fluorescence properties of the pyrene-labeled PNA is sensitive to its hybridization state and can readily distinguish between complementary and single-mismatched DNA targets and can used as the tool for DNA sequence determination.