CHAPTER IV

CONCLUSION

The reductive alkylation of proline derivatives, modified by nucleobases (adenine, thymine, cytosine and guanine) at C-4 position in a *cis*- and *trans*-relationship to the carboxyl group ("*cis*-D" or (2*R*,4*S*), "*cis*-L" or (2*S*,4*S*), "*trans*-D" or (2*R*,4*S*) and "*trans*-L" or (2*S*,4*R*)), is a more efficient route for synthesis of *aep*PNA monomer than the previously reported methods [44-47]. It provides the Fmoc derivatives directly without the need for protecting group conversion. All *aep*PNAs (**23-29**) was synthesized by coupling of the proline derivatives (**6a**, **6b**, **6c**, **6d**, **7**, **8**, **9**), after removal of the N-terminal Boc protecting group, with Fmoc-aminoacetaldehyde (**19**) in the presence of $NaBH_3CN$ as the reducing agent and NaOAc as a buffer. This reaction take place without epimerization at the position 2' in proline ring as determined by ^1H NMR spectrum.

Selective deprotection of the C-terminal protecting group followed by activation with PfpOTfa/DIEA gave the desired *cis*-D *aep*PNA monomers containing all four nucleobase (A^{Bz}, T, C^{Bz} and G^{Ibu}) and thymine monomer with different stereochemistry (*cis*-D, *cis*-L, *trans*-D and *trans*-L). Oligomerizations were carried out employing Fmoc SPPS coupling strategy. Six *aep*PNA decamers (**44-49**) were successfully synthesized. These were purified by reverse phase HPLC and characterized by MALDI-TOF mass spectrometry.

The hybridization property of the *aep*PNA was investigated by UV melting experiments. *Cis*-D and *cis*-L homothymine *aep*PNA decamers (**44**, **45**) formed stable hybrids with poly(rA) (T_m 42 and 43 °C) but they could not form hybrids with poly(dA). On the other hand, *trans*-D homothymine *aep*PNA decamer (**46**) failed to form stable hybrid with poly(rA) but it formed a rather unstable hybrid with poly(dA) (T_m of 24 °C). *Trans*-L homothymine *aep*PNA decamer (**47**) neither bound to DNA nor RNA, whereas *cis*-D homoadenine *aep*PNA decamer (**48**) bound to both poly(dT) and poly(rU) with T_m 21 and 23 °C, respectively. Therefore, the difference of stereochemistries on the pyrolidine ring and nucleobase sequence can have a dramatic effect on the binding characteristics of the *aep*PNA. In case of UV titration between

cis-D homothymine *aep*PNA decamers (**44**) and poly(rA) shown a 2:1 stoichiometry of T:A, indicating the formation of a triple helical complex, probably *via* Watson-Crick and Hoogsteen-type T·A·T pairing similar to that according to the results of Vilaivan *et al.* [47].

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย