CHAPTER I

INTRODUCTION

1.1 Literature reviews

It was proposed and has been experimentally demonstrated that synthetic oligonucleotides may be potentially useful for therapeutic applications.[1] According to this hypothesis, they would bind to mRNA specifically and inhibit protein synthesis by translation process which would thus control genetic expression. This principle is well-known as "antisense principle" and the synthetic oligonucleotide capable of controlling genetic expression is called "antisense oligonucleotide".[2] In 1978, Zamecnik and Stephenson first used this approach to inhibit cancer formation by Rous Sarcoma Virus with synthetic oligonucleotides.[1] Many diseases originates from malfunctioning of enzyme or proteins resulting from mRNA translation. Hence inhibition at mRNA level should, in principle, be more effective than inhibition at enzyme or protein level. This principle has been extended to inhibit transcription process of DNA into mRNA which believed to be even better than mRNA translation inhibition and thus named as "antigene principle" (figure 1.1).[3]

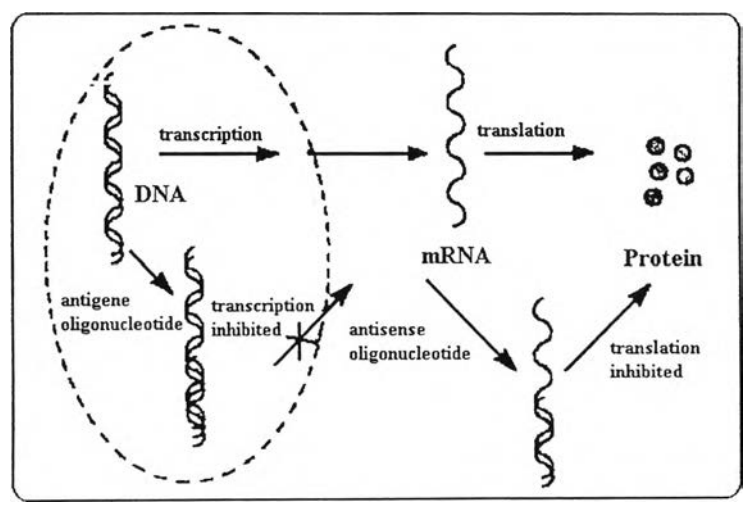


Figure 1.1 A schematic diagram illustrates the gene expression inhibition by antisense or antigene approach

It was proposed that the antisense or antigene oligonucleotides would bind to mRNA or DNA relying on the highly specific Watson-Crick base pairing (figure 1.2) and this makes them more attractive than the classical drug in term of specificity to the targets of diseases. Any desired sequence of antisense oligonucleotides could be easily synthesized when the complementary sequence of the target nucleic acid was known. The feasibility of putting antisense approach into practice is strengthened by the fact that there is no unrecurred sequence of 12-mer appear statistically in three billion pairs of nucleotide bases in human genome when all four nucleobases are present in an equal number and randomly distributed throughout the gene.[4] Therefore antisense oligonucleotide would show specific inhibition even when only short sequence is used. Interestingly, it was pointed out that the antigene approach seemed to be more effective than the antisense, based on the fact that mRNA was found in many cellular organelles whereas DNA was template for mRNA and was only limited in nucleus. As mentioned above, the antisense and antigene approach have opened up a new way to develop rational drug design and become one important candidate for treatment of genetically related disease.

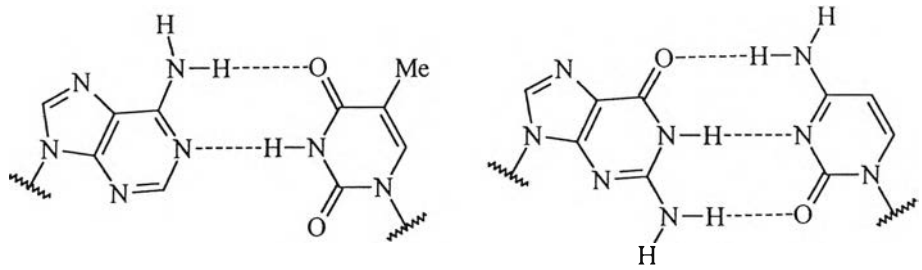


Figure 1.2 Watson-Crick base pairing A•T and C•G

1.1.1 Modified Oligonucleotides: A step towards antisense drugs

In order to apply the antisense principle successfully in practice, the antisense oligonucleotide must meet the following basic requirements[2]: 1) the complex formed between the oligonucleotide and its complementary sequencene target must be sufficiently stable under physiological conditions, 2) the interaction between the oligonucleotide and its nucleic acid target must be specific, 3) the hybrid formed must be stable enough to inhibit the normal action of target nucleic acids, and 4) the antisense oligonucleotides

must be able to pass through the cell membrane or nucleus membrane to reach the nucleic acid target. Disappointingly, even when specific binding between the antisense oligonucleotide and nucleic acid target has proven to be promising, it was limited by their inherent susceptibility toward cellular nuclease and their poorly transported across the cell membrane due to electrostatic repulsion of the intrinsic anionic nature of the phosphodiester to polyanionic cell membrane. Accordingly, the antisense oligonucleotides must be modified in order to overcome this problem.

The first generation of modified oligonucleotides developed contain the phosphodiester linkages with one or more oxgen atoms were replaced by appropriate atoms or groups such as hydrogen,[5] sulfur, nitrogen,[6] boron, alkyl group, substituted amino group to give modified phosphorus-containing oligonucleotide (figure 1.3 a).

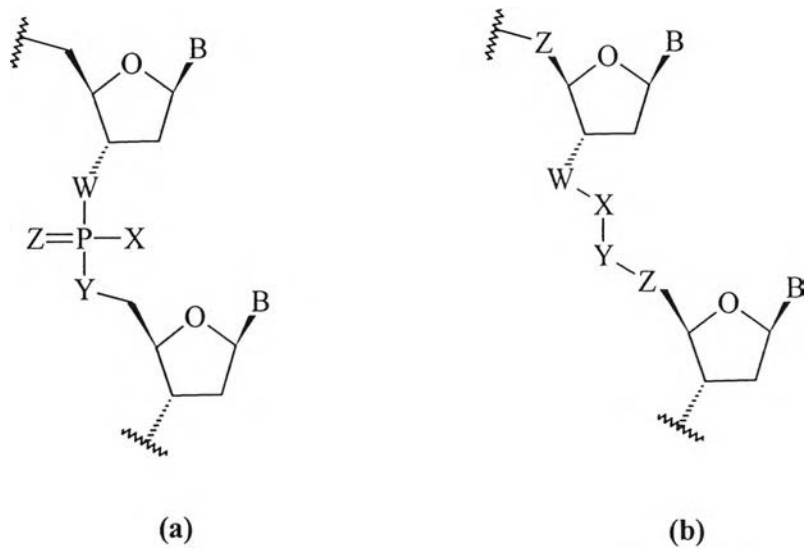


Figure 1.3 Structures of modified phosphorus-containing oligonucleotides (a) ; and dephospho-oligonucleotides (b) where w, x, y and z represented substituted atoms in the phosphodiester linkage

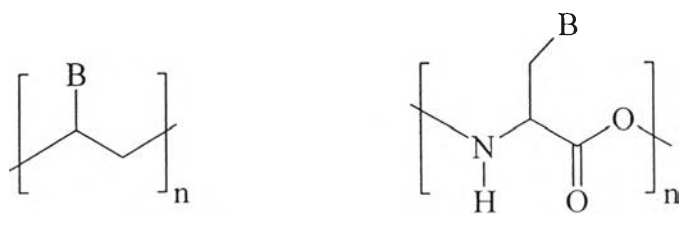
It was believed that the increased affinity because of such modification came from the lack of electrostatic repulsion of their uncharged backbone to negative charge of phosphodiester which serve to stabilize the complex.[7] However, except for phosphothioate and methylphosphonate oligonucleotides, other modifications gave poorer binding result than unmodified oligonucleotide. Even though these modified oligonucleotides displayed a

greatly improved resistance towards nucleases and exhibited high binding affinity, these replacement brought about diastereomeric mixture when only one oxygen atom in nonbridging phosphodiester was replaced. Two possible stereoisomers were obtained for each modified unit and resulted in 2^n diastereomers (where n = number of modification) for oligomer containing n modified units.

One way to circumvent the problem arising from stereochemical inhomogeneity is to replace the entire phosphate group with other spacer while still retaining the proper distance in the phosphodiester bridge. The resulting oligonucleotide was called "dephospho–oligonucleotides" and included modification such as carboxylic ester, carbamate, carbonate, sulfide, sulfone,[2] amide,[8] formalacetal [9] (figure 1.3 b). Although this replacement did not provide diastereomeric effect and the resulting oligonucleotide could tolerate the nuclease degradation, most of them gave poorer binding affinity than unmodified oligonucleotide and poor water solubility due to hydrophobic properties of the linker.

1.1.2 Backbone modification

A rather radical modification of oligonucleotide involves abadoning the sugarphosphate backbone altogether. This modification has advantages over the previous modified oligonucleotide because the design to give the desired properties seemed to be easier while the binding affinity towards the natural oligonucleotides was still retained. Although many backbone modification have been reported in literatures since 1960 such as plastic DNA,[10] biopolymer carrying nucleobase or willardiine,[11] the spacing between adjacent nucleobase was considered to be far too short to allow the hybridization with complementary oligonucleotide (figure 1.4).

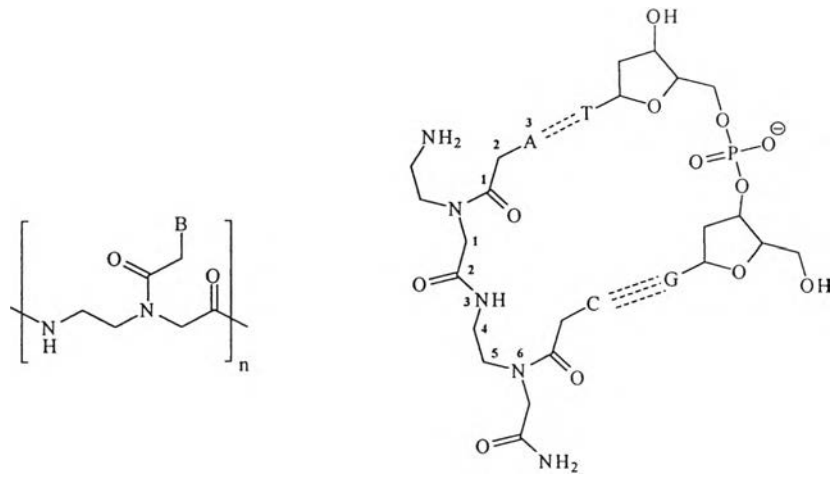


Plastic DNA **Willardiine**

Figure 1.4 Some modified backbone oligonucleotides where B= nucleobase

1.1.3 Peptide Nucleic Acid (PNA)

These radical backbone modifications brought about a new concept to construct the molecule which could recognize and bind stably to genetic material and was not substrate for nuclease. In 1991, P. E. Nielsen and co-worker at the Unversity of Copenhagen have published pioneer reports on discovering a new DNA analog in which the entire ribose phosphate is replaced with *N*-(2-aminoethylglycine) unit and the nucleobase was attached to the central amine in backbone *via* acetyl linker (figure 1.5 a).[12] Since this DNA homomorphous structure was derived from polyamide and might be considered as polypeptide, hence it was named as "Peptide Nucleic Acid" or "Polyamide Nucleic Acid" and short in "PNA".[12] PNA has the number of atoms between the ends of one monomeric unit being the same as in DNA. The distance between nucleobase and backbone was 3 atoms while each central amino group is 6 atoms apart. This relationship was established as (6+3) rule (figure 1.5 b).[12]



(a) (b)

Figure 1.5 Structures of Peptide Nucleic Acid (PNA) (a) and 6+3 rule (b)

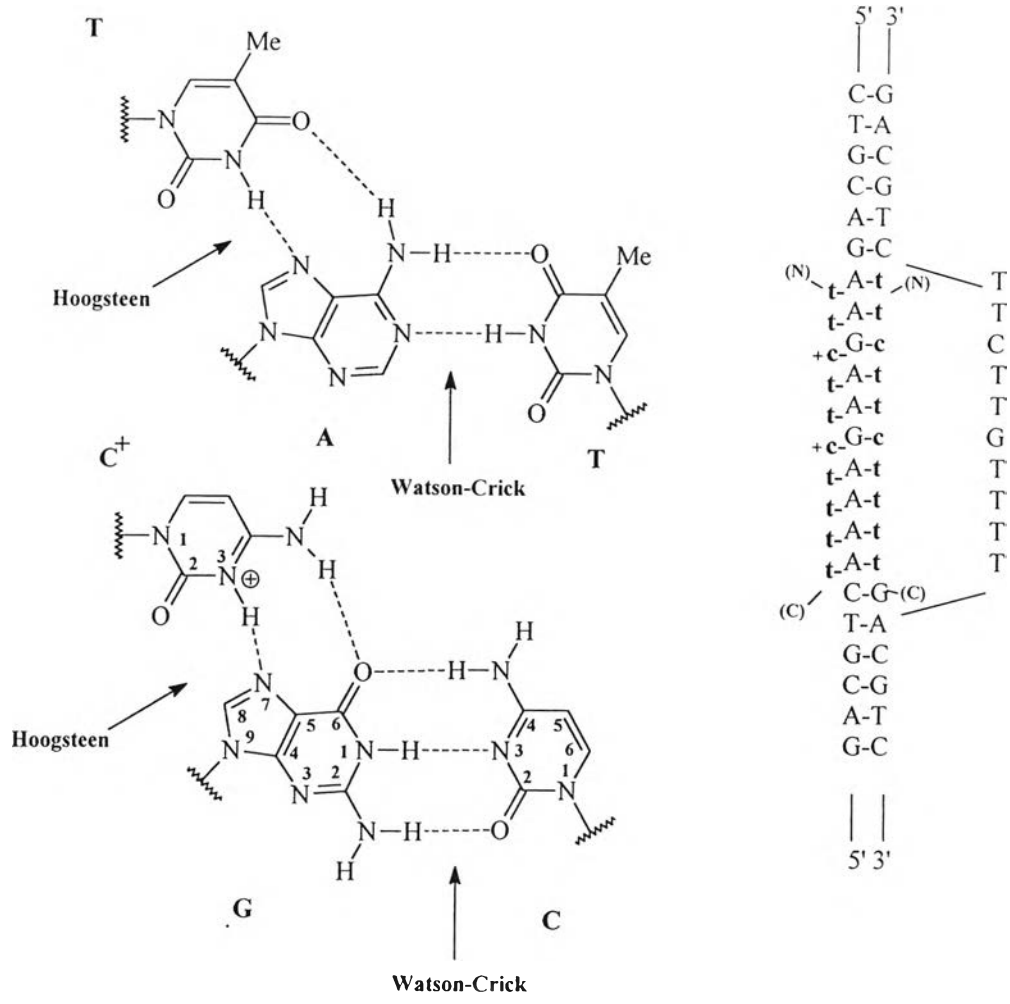
PNA could be easily synthesized and did not give the diastereomeric mixture since it is achiral. It was surprising to find that PNA could form a remarkably stable complex with complementary oligonucleotide as evidenced from the melting temperature (T_m) of 73 °C of the complex formed between PNA 10-mer carrying thymine (t_{10}PNA) and

complementary oligonucleotide (dA_{10}) whereas the corresponding oligonucleotide complex, (dA_{10}).(dT_{10}) melts at room temperature.[13] It was believed that the major reason for such stabilization resulted from the lack of electrostatic repulsion due to the uncharged backbone of PNA. In addition the suitable proper base to base distance and partial flexibility of the backbone may also play an important role. The sequence specificity of base pairing was shown by a sharp decrease in T_m of 13 °C when a single mismatch was introduced.[13]

1.1.4 PNA hybridization and their secondary structures

Hybridization between homopyrimidine PNA and homopurine targets in double strand DNA did not result in a conventional PNA(DNA)$_2$ triplex under the AA*T and GG*C base motif which usually found in DNA strand displacement, but (PNA)$_2$DNA triplex was formed as indicated by UV-titration confirmed the 2:1 stoichiometric proportion PNA:DNA.[14,15] The first PNA strand invaded into the major groove of double strand DNA *via* formation of a transient PNA/DNA Watson-Crick duplex, which is subsequently trapped by a second PNA, forming Hoogsteen base pairing with the homopurine target through the minor groove and then irreversibly converted into a stable (PNA)$_2$DNA structure known as "P-loop". In such triplex structure, the N^3-position cytosine of Hoogsteen PNA strand was necessarily protonated (figure 1.6).[16,17,18]

Both PNA strands in P-loop were laid down in parallel orientation (N-terminus of PNA facing 5'-OH of DNA).[19] The preference of (PNA)$_2$DNA triple helix formation has been supported by molecular mechanic calculation, comprizing of intra- and inter-molecular hydrogen bonding.[20,21] Although formation of intramolecular hydrogen bonding (between NH-glycyl amide proton and acetyl linker carbonyl) would seem to be reasonable, only intermolecular hydrogen bonding between each amide NH and phosphate oxygen of backbone was observed in practice as confirmed by X-ray crystal structure,[22] and NMR spectroscopy.[23] Kinetic and mechanism of P-loop formation have been studied by gel retardation and nuclease S1 cleavage assay which revealed a pseudo-first order kinetic.[24]



(a) (b)

Figure 1.6 Hydrogen bonding *via* Watson-Crick and Hoogsteen base pairing (a); "P-loop" triple helix formation of $(PNA)_2DNA$ (b) where A, T, C, G represent DNA nucleobase, **t** and **c** represent PNA nucleobase in P-loop, **(N)** and **(C)** were the N-terminus and C-terminus of PNA respectively

The possible mechanism of complex formation was proposed to involve: i) a transient partial opening of the PNA binding site on double strand DNA and incorporation of one PNA molecule with formation of an intermediate PNA/DNA duplex (DNA breathing) and ii) formation of a very stable PNA_2/DNA triplex.[24] Duplex forming between homopurine PNA (A_4G_2AGAG) to a complementary homopyrimidine target in double strand DNA was found to proceed through the duplex invasion to form $PNA(DNA)_2$ (figure 1.7 b). Preliminary studies revealed that this complex could be in

either of parallel and antiparallel mode, but antiparallel binding orientaion of two purine strands (N-teminal PNA facing 3′-OH DNA) gave a higher T_m value.[25] Cytosine rich homopyrimidine PNAs bound to guanine strand of double strand DNA and become triplex depending on N^3-protonation and Hoogsteen base pairing without strand displacement (figure 17 d).[16] Complexation of PNA to DNA could be summarized in figure 1.7.

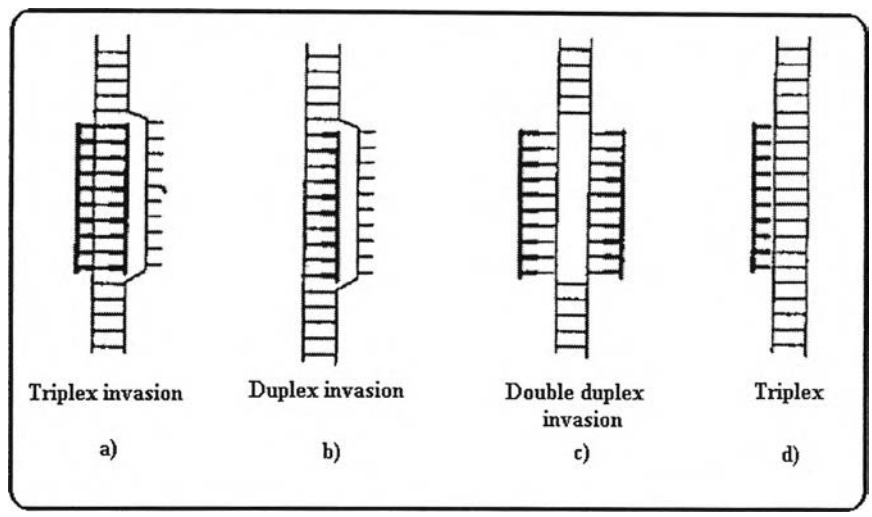


Figure 1.7 Schematic drawing of complexes formed upon targeting double strand DNA with PNAs (bold): (a) triplex invasion complex is the most stable and is formed P-loop; (b) duplex invasion complex can be formed by homopurine PNA; (c) double duplex invasion complex requires non-natural nucleobases in the PNAs strand; (d) a conventional triplex seems to be formed only with cytosine rich homopyrimidine PNA

Hybridization of PNA to RNA has been studied by NMR spectroscopy.[26] The result showed a hexameric PNA formed a 1:1 complex with complementary RNA to give an antiparallel, right-handed double helix with Watson-Crick base pairing. PNA-PNA hybridization of orginal PNA and cyclohexyl modified PNA has been studied by melting temperature, CD titration and CD spectroscopy.[27] Homothymine 10-mer PNA could form triple helix to homopurine PNA with 2:1 stoichiometry and gave a higher melting temperature (76°C) comparing to PNA-DNA (71.5°C) under the same condition. In addition, replacing some sequence original PNA with cyclohexyl PNA has resulted in right

handed helix when *R,R* configuration of cyclohexyl moiety was introduced but the *S,S* configuration gave opposite results. The hybridization stability of PNA to complementary nucleic acid target might be arranged according to melting temperature as follows: PNA-PNA >PNA-RNA >PNA-DNA > (RNA-DNA >DNA-DNA). The secondary structure of PNA-PNA duplex possessed a very wide diameter (28 °A) and has a large pitch (18 base pairs per turn) while the B-form of DNA was *ca* 20 °A, 10 base pairs per turn and A-form was *ca* 20 °A, 11 base pairs per turn.[22] From all above, PNA oligomer is, to a great extent, able to conformationally adapt to its more rigid oligonucleotide complement. Three dimentional of PNA complexed with complementary oligonucleotides are summarized in figure 1.8.

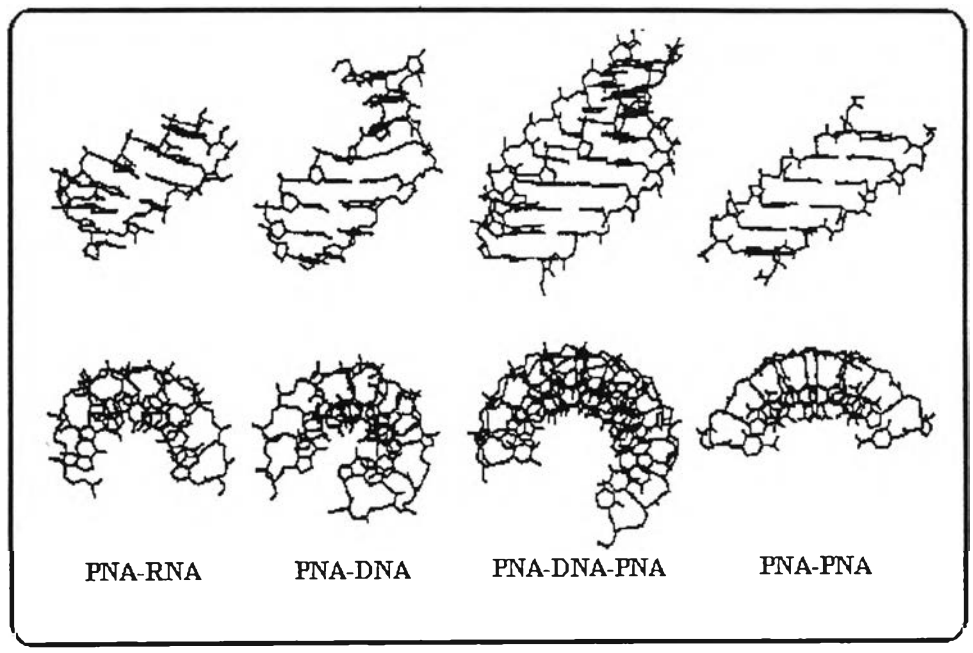


Figure 1.8 Three dimentional structures of PNA complexes; perpendicular view (lower) and along the helix axis view (upper)

1.1.5 PNA structural modifications

The success of PNA attracted much attention from many research groups to modified the original PNAs to meet the desired properties to be drug candidates. Extending the PNA backbone or nucleobase acetyl linker with a methylene subunit, generated *N*-(2-aminoethyl-β-alanine) (βB), *N*-(3-aminopropyl)glycine (apgB) and propionic acid linker

(PaT) (figure 1.9 a). These extened PNA lost their binding affinity to complementary oligonucleotides; even though the base to backbone extending could preserve the binding specificity.[28] Replacing the acetyl linker with more flexible ethylene linker caused decremental in affinity also (figure 1.9 b).[29] Influence of rigidity in PNA backbone have been studied by Krotz who reversed the amide bond of original PNA into reversed amide PNA and "retro-inverso" PNA (figure 1.9 c). By this modification, the distance between nitrogens to which the nucleobase moieties are attached is six bonds and obeying 6+3 rule,[30] but their binding stability was still decreased.[22] Similar idea was made by moving carbonyl group of acetyl linker into the aminoethyl part of PNA backbone which serves to enhance the rigidity of PNA backbone (figure 1.9 d), but this PNA 9-mer carrying thymine lost all binding affinity to its complementary oligonucleotide.[30]

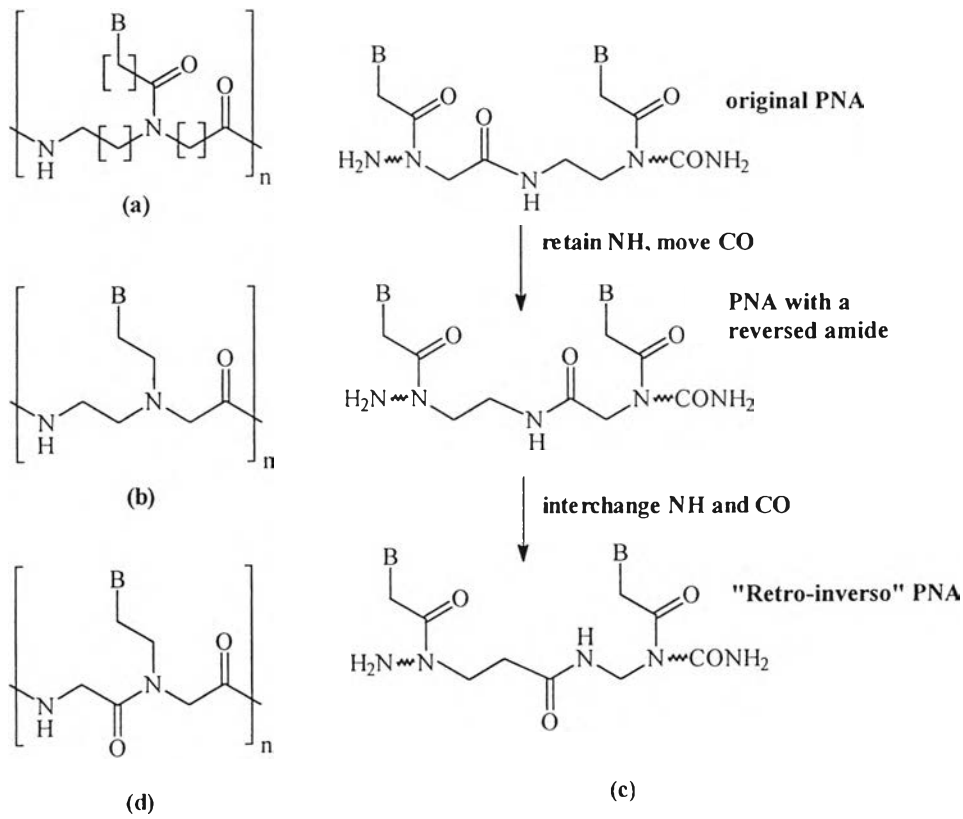


Figure 1.9 Structures of extended modified PNAs (a); PNA with ethylene linker from base to backbone (b); reversed amide PNA and "retro-inverso" PNA (c); PNA with moving carbonyl of acetyl linker to backbone (d)

Incorporation of some non-natural nucleobases into PNA have been reported to improve binding stability and specificity. 3-Oxo-2,3-dihydropyridazine was used to

recognized thymine target strand in double strands DNA (figure 1.10 a).[31] The pH dependence of $(PNA)_2DNA$ triple helix could be eliminated by incorporation of pseudoisocytosine (equivalent to permanently protonated cytosine) in place of cytosine in the Hoogsteen strand (figure 1.10 b).[30] Similarly, diaminopurine could recognize thymine with three hydrogen bonding (usually A•T is two hydrogen bonding) and thus increase the stability of PNA complexes (figure 1.10 c).[32] Other non-natural nucleobase systems are shown in figure 1.10.

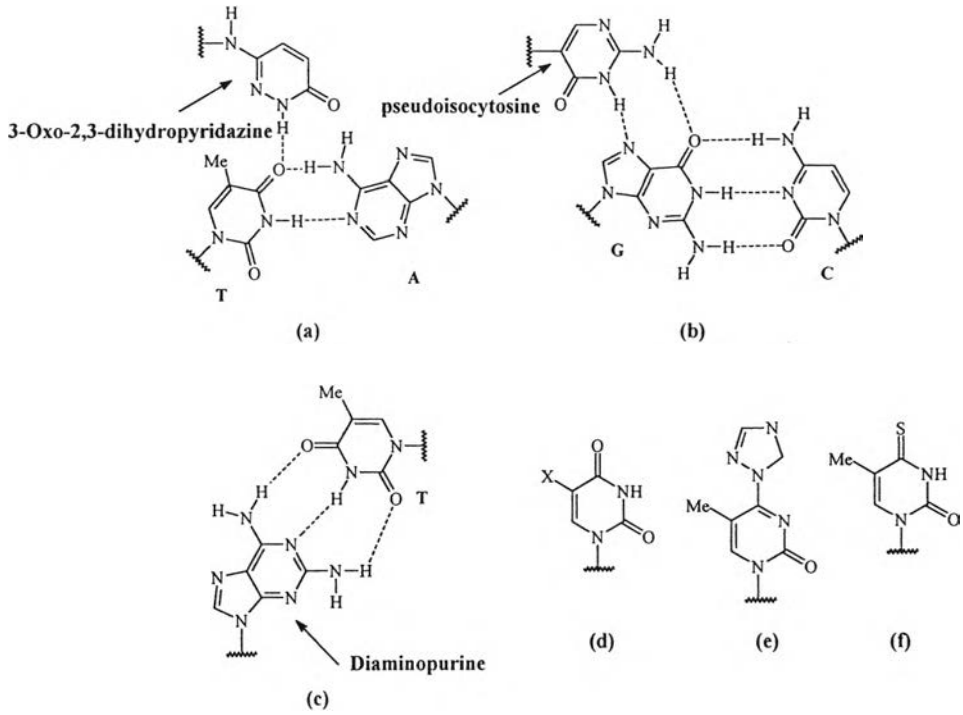
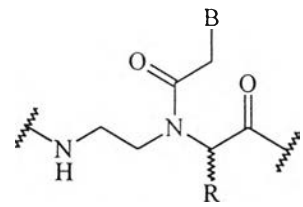


Figure 1.10 Structures of non-natural nucleobases (a) 3-oxo-2,3 -dihydropyridazine; (b) pseudoisocytosine; (c) diaminopurine; (d) 5-bromo- (X = Br) or 5-Iodo-uracil (X = I); (e) triazolothymine; (f) 4-thiothymine

Further modification was introducing chirality into the original PNA in order to increase the binding properties and selectivity. Replacement of the glycine unit in PNA building block by other amino acids have originated the chiral PNA or short in "cPNA" with varying in degree of binding stability depending on the nature of the side chain (Table 1.1).[22] It was evidenced that stabilizing effect was obtained when positively-charged side chains such as lysine or uncharged side chains were used while the negatively-charged side

chains gave destabilizing effect. Interestingly, D-amino acid always gave T_m higher than L-amino acid.

Table 1.1 Effects on thermal stability per monomer (ΔT_m/°C) for the PNA sequence H-gtagatcact-NH_2 incorporating three chiral monomers as compared to unmodified PNA[22]



R	Chirality	ΔT_m DNA/°C	ΔT_m RNA/°C
CH_3	L	-1.8	nd
CH_3	D	-0.7	nd
^sBU	L	-2.6	-3.0
$CH_2(OH)$	L	-1.0	-1.0
$CH_2(OH)$	D	-0.6	-1.0
CH_2CO_2H	L	-3.3	nd
$CH_2CH_2CO_2H$	D	-2.3	nd
$(CH_2)_4NH_2$	L	-1.0	-1.3
$(CH_2)_4NH_2$	D	+1.0	0

Introducing chirality might proceed simultaneously with relocating the nucleobase linker, this was found in replacing the aminoethyl moiety and acetyl linker with homoserine to generate "αPNA" (figure 1.11 a).[33] The nucleobase was positioned on homoserine side chain which the proper distance was still retained and obeying 6+3 rule. αPNA containing N^{β}-(cytosine-1-yl acetyl)-β-aminoalanine showed lower binding stability comparing to the original PNA even when the structure was designed so as to form intra- and inter-residue hydrogen bonding (figure 1.11 b).[34] Similar, ($SerCH_2B$) glycine backbone modified PNAs were synthesized but their properties have not been reported (figure 1.11 c).[35] Nucleobase relocation and replacement ether linkage instead of central amine of orginal PNA has resulted in "oxy-PNA" or "OPNA" with the hope to improve

water solubility. The binding affinity of OPNA was found to be all-or-none hybridization type and was sequence specific (figure 1.11 d)[36] and this perhaps is one of the most successful modification of PNA reported to date.

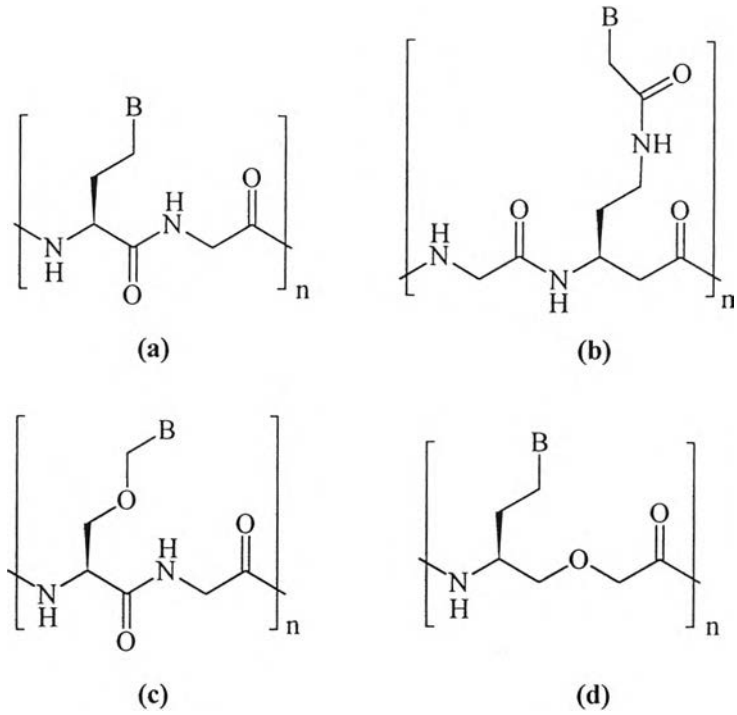


Figure 1.11 Structures of some chiral PNAs

Thermodynamic consideration has suggested that the hybridization of PNA to complementary oligonucleotide caused the enthalpy gain and significant entropy loss due to both PNA and DNA structures were flexible prior to binding altogether to form a more rigid complex. The free energy gain of complex formation may be decreased by reducing the entropy loss. One way to solve this problem is by locking the conformation of PNA to be the same as in the rigid complex.[22] For this reason, many researchers have paid attention focusing on structural preorganization of modified PNA such as introducing rigid backbone to form conformationally constrained PNA. The central tertiary amine bond of original PNA was conceptually fixed by replacing the acetyl linker with a Z-olefinic group (figure 1.12 a). This study confirmed that all amide bonds connecting the base linker to backbone are uniformly oriented in Z-form within duplex but was E/Z equilibrium in free PNA strand.[38] PNA carrying chiral alanylproline backbone in which the nucleobase was

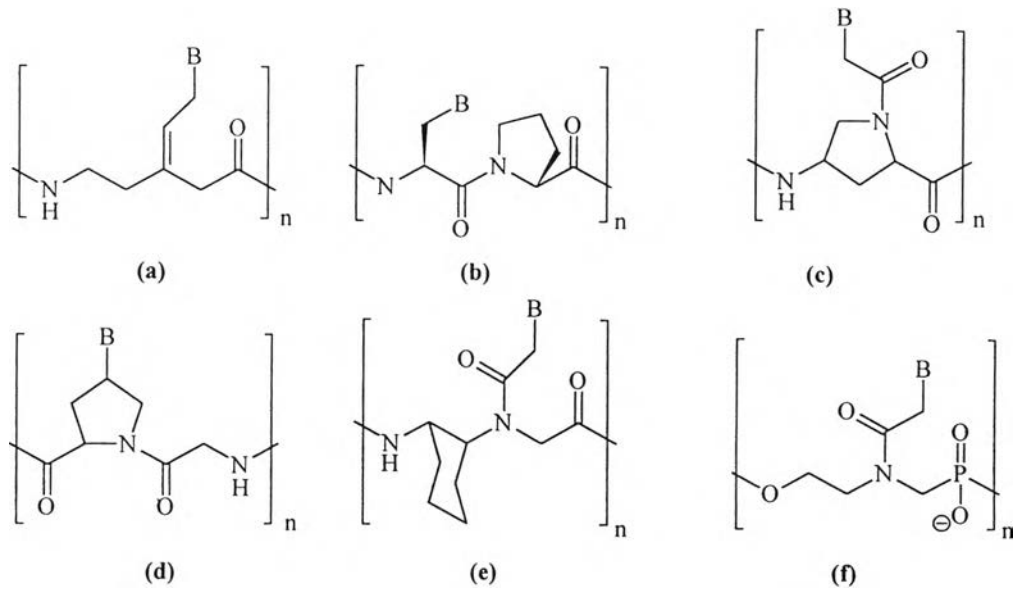


Figure 1.12 Structures of some conformationally constraint PNAs and PHONA

attached to the alanine side chain have been synthesized (figure 1.12 b).[39] PNA based on 4-aminoproryl backbone with two chiral centers at C-2 and C-4 have been synthesized (figure 1.12 c).[40] Homothymine hexamers of three diastereomers (*trans*-D/L, *cis*-L) failed to form any complexes with dA_6. Conversely, insertion of such PNAs unit either at the N-terminus or within the strand of the original PNA resulted in oligomers which are able to form complexes with complementary oligonucleotide in both parallel and antiparallel orientation with higher T_m compared to the original PNA.[41] An other interesting proline system of *cis*-L and *trans*-L isomers of both 4-aminoproline and pyrrolidine-2-carboxylic acid derivative carrying nucleobase at C-4 position were studied which could not form the stable complexes (figure 1.12 c and d).[42] However, homothymine of these 4-6 mer PNA alternating sequence with original PNA have higher the melting temperature than original PNA about 6-7 °C when the configuration on proline was *trans*-L.[43] Modified PNA containing glycylproline as a monomeric building block with *cis*-configuration has been synthesized (figure 1.12 d).[44,45,46] Homothymine 10-mer of *cis*-L and *cis*-D series could bind strongly to poly dA as indicated by T_m of 69 and 70 °C respectively whereas their T_m with to poly rA was 73 and 72 °C respectively.[47] The PNA deriving from (*R,R*) and (*S,S*) cyclohexyl backbone were also reported (figure 1.12 e).[48] The binding stability has

diminished (- 1 °C) when (*S,S*) configuration was introduced. A new hybrid molecule combining both original PNA and DNA was synthesized in order to improve water solubility and cellular permeability.[49] This hybrid was known as "PNA/DNA chimera" Replacing glycyl amide bond in PNA with phosphonic acid ester bridge has generated a new PNA with negatively-charge and was called as "PHONA".[50] The mixed sequence containing PHONA and original PNA was shown to improve water solubility and binding affinity (figure 1.12 f).[50]

1.1.6 PNA applications

PNAs have great the potential to be developed into antisense and antigene drugs or other related application relying on their binding stability and sequence specific properties. The t_{10}PNA or mixed sequence 15-mer PNA could bind to its complementary target of template strand in double strand DNA and could arrest the transcription by RNA polymerase (figure 1.13 a). On the other hand, binding of PNA to complementary target in non-template strand has success with only 50% efficiency.[51] PNAs were not only transcription inhibitors but may also be activator. The $(PNA)_2$DNA strand displacement complex could be recognized by RNA polymerase and initiated PNA transcription at an efficiency comparable to that of the strong *E.Coli* lac UV-5 promoter. Thus this PNA activator might be considered as "artificial transcription promoter" (figure 1.13 c).[52] PNA-DNA chimeras have been used as DNA primer to support the DNA synthesis catalyzed by *E.Coli* DNA polymerase I.[53] Plasmid containing double stranded 10-mer PNA/DNA chimeras showed sequence specific inhibition to DNA restriction enzyme.[54] PNAs could inhibit translation specifically when these PNAs were targeted against the 5′-proximal region to the AUG start codon, whereas targeting on coding region are inactive.[29] Normally, an exclusive PNA did not stimulate the RNase H enzyme to cleave its complementary RNA as good as the natural oligonucleotide, but the PNA/DNA chimeras are able to stimulate the RNase H and thus cleavage of RNA at DNA part of chimera (figure 1.13 d).[29]

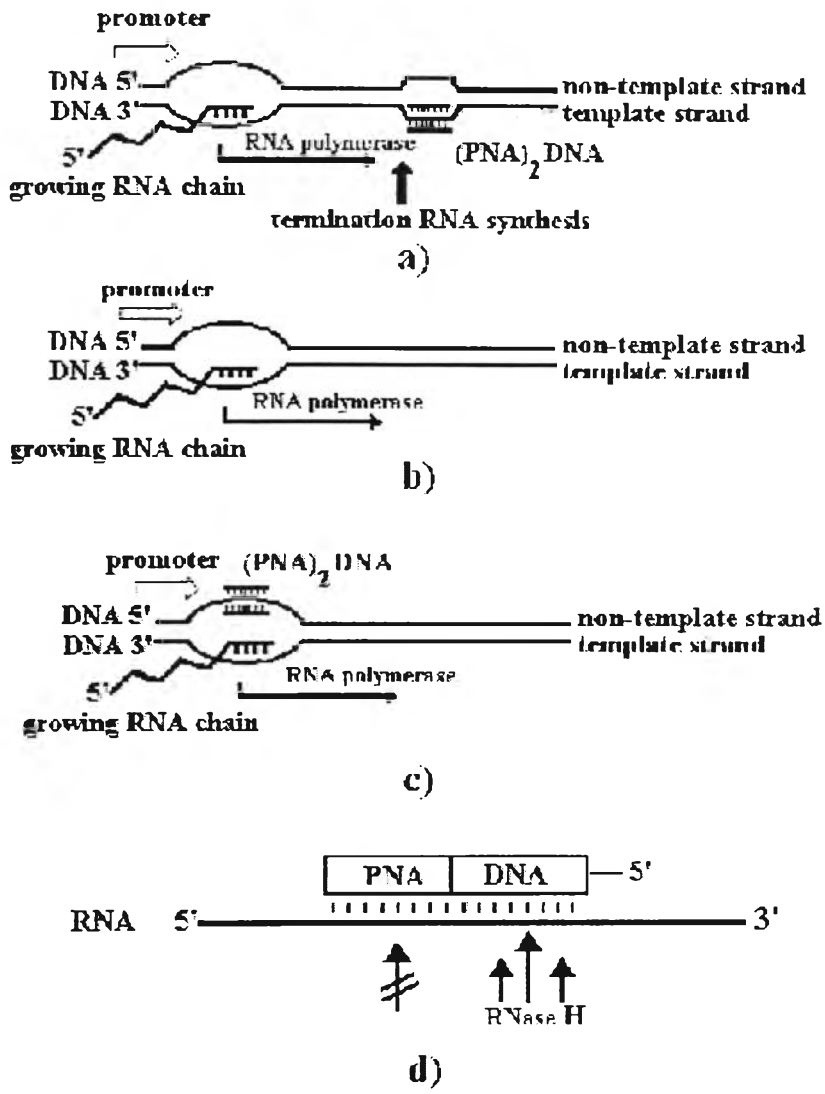


Figure 1.13 Effect of PNA to RNA transcription (a) inhibition of transcription by PNA through strand invasion and triple helix formation on the single strand; (b) open promoter complex; (c) activation of transcription by PNAs; (d) RNase H-mediated cleavage of RNA in a duplex with a PNA/DNA chimera

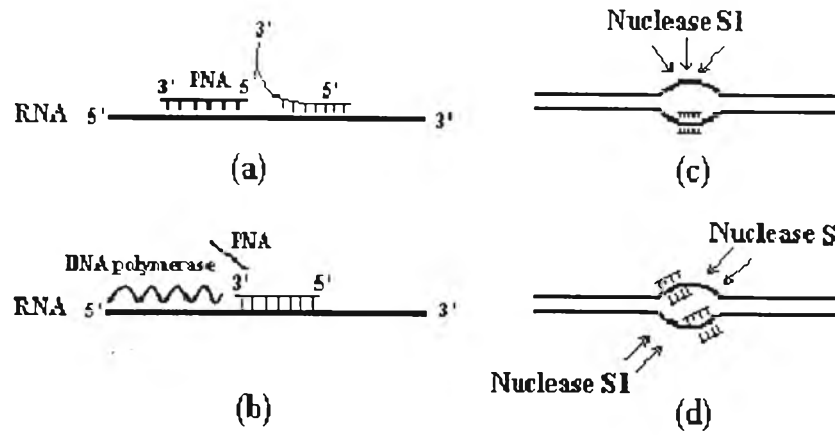


Figure 1.14 Some applications of PNA in diagnostics and biotechnology: (a) blockage of the PCR reaction by strong binding of the PNA to the wild-type gene; (b) positive PCR caused by weaker PNA binding to the mutated gene; (c) single strand cleavage by artificial restriction enzyme; (d) double strand cleavage by artificial restriction enzyme

PNAs have also been applied to diagnostics such as screening for genetic mutation (CFTR gene in cystic fibrosis),[29] detection of point mutation ("PCR clampling").[29] (figure 1.14 a and b). In addition, binding of short sequence PNAs to double strand DNA at the undesired cleavaged region converted it into local $(PNA)_2DNA$ triple helix which prevented cleavage of the DNA strand by single strand specific nuclease S1 (figure 1.14 c and d).[55] This combination of PNA and single strand nuclease S1 could provide the result as if they were a restriction enzyme, hence they were named as "artificial restriction nuclease".[55] Another method for selective cleavage involving PNA was achieved by protection the desired cleavage region on DNA with PNA, methylated the free hydroxyl group of the ribose and phosphodiester linkage in DNA with methylase in order to prevent DNA cleavage and then the PNA removed followed by cleavage of the DNA strand in the PNA-free region without disturbing the methylated region. This technique was known as "Achilles heel approach" [56] or "genome rare cutter" (figure 1.15).[57]

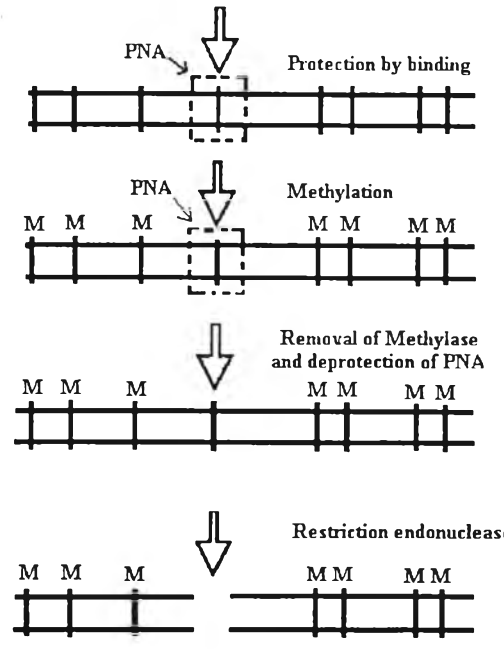


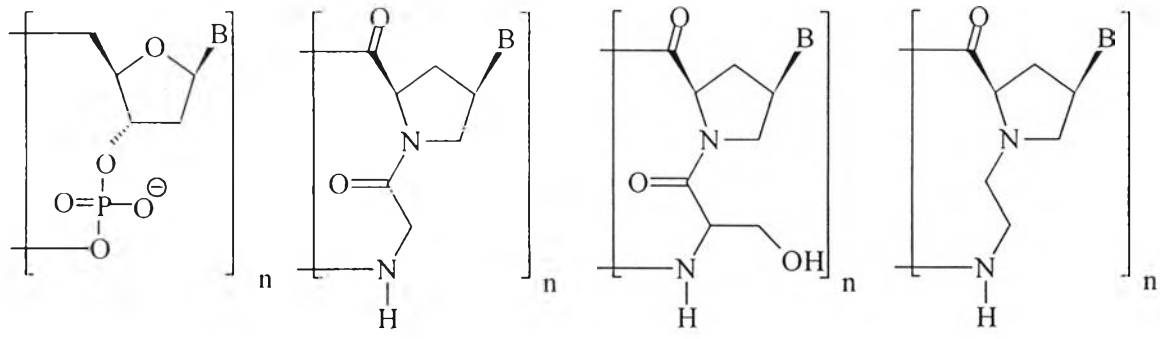
Figure 1.15 Schematic of the "Achilles heel" approach or genome rare cutter

1.1.7 Problems of PNA and their solutions

The major problems of PNA application were low water solubility and cellular permeability, therefore no biological activity of pure PNA have been reported so far without the use of other techniques that serve to bypass the cell membrane. PNA 10-mer could diffuse passively through the phospholipid membrane (liposome) with a halflife of 11 days while those of similar natural oligonucleotide was 7 days.[58] Connecting polylysine to the C-terminus of PNA has greatly improved water solubility, but the solubility decreases as the length and purine:pyrimidine ratio increases.[29] Other popular methods to convey PNA into cells reported included conjugating with biotin[59] or lysolecitin,[29] using some detergents like tween-20, covalent attachment with some peptide binding selectively to cell surface receptor and direct microinjection for free cell system.[29] Surprisingly, there have been only a few literatures expressing some interests to solve these problems by modifing PNA structures so that modified PNAs would pass into the cell and dissolve in water easily. It most cases such modified PNA always have negatively-charges such as PHONA and PNA/DNA chimera[49,50] or neutral such as OPNA.[36]

In 1997, a research group at University of Oxford, has reported a novel PNA which derived from glycylproline backbone, linked to nucleobase at proline C-4 position (figure 1.16 a).[44,45,46] The resulting PNA was conformationally constraint PNA and should, in principle, discriminate parallel and antiparallel orientation better than achiral PNA. Preliminary binding stabilities of these cPNAs were studied which shown the high stability as good as original PNA when proline ring have "*cis*-D"configuration. Preliminary experiments have indicated that the interaction between homothymine 10-mer of *cis*-D-isomer (CD-T10G) and dA_{10} would probably be specific for A•T pair but this is yet to be confirmed by other techniques. Unfortunately, the solubility of the cPNA and their hybrids were not sufficient to allow measurement with ^1H NMR and would almost certainly have effect to cell permeability.

It was decided to modify the glyclyproline cPNA having "*cis*-D" configuration in order to increase its solubility. Two strategies were employed for this purpose. First, the glycine spacer was replaced with serine which possesses alcoholic side chain to form serylproline cPNA (figure 1.16 b). Serine contains one chiral center and thus may exist as L- or D-form. It was therfore necessary to synthesis and study binding stabilities of both isomers. Second, by reducing the amide bond between glycine and proline to generate a deoxyglycylproline cPNA (figure 1.16 c) with the hope that nitrogen of which proline ring (pK_a~8) would be partially protonated as ammonium salt at pH~7. This positive charge might serve to increase the solubility and would also stabilize the complex formation *via* electrostatic attraction to the negative charges of natural complementary oligonucleotide. In addition, changing the sp^2 hybridization of amide into sp^3 in amine caused to enhance the flexibility of cPNA backbone which could make it adopt the B-DNA like conformation more easily.



DNA **(a)** **(b)** **(c)**

Figure 1.16 Structures of DNA; glycylproline cPNA (a); serylproline cPNA (b) and deoxyglycylproline cPNA (c)

1.2 Objectives of this research

The objective of this research is to synthesize novel chiral peptide nucleic acids (cPNA) carrying either L, D-serine and aminoethyl spacers and to study their binding properties with complementary unmodified oligonucleotides.