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**SYNTHESIS AND NUCLEIC ACID BINDING PROPERTIES
OF NOVEL PEPTIDE NUCLEIC ACIDS
CARRYING BETA AMINO ACID SPACER**

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**A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Chemistry**

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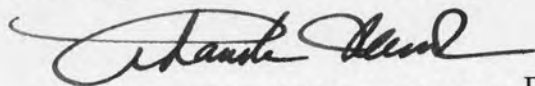
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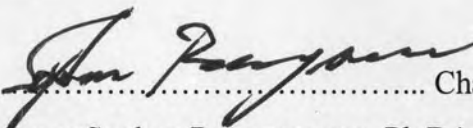
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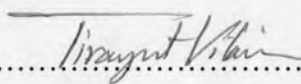
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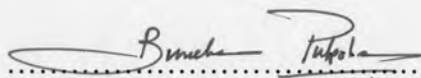
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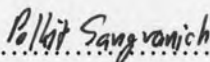
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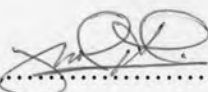
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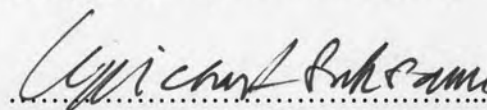
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จตุรงค์ สุภาพพร้อม : การสังเคราะห์และสมบัติการจับยึดกับกรดนิวคลีอิกของกรดนิวคลีอิกเพปไทด์ชนิดใหม่ที่มีสะพานเชื่อมเป็นกรดบีตาเอมิโน (SYNTHESIS AND NUCLEIC ACID BINDING PROPERTIES OF NOVEL PEPTIDE NUCLEIC ACIDS CARRYING BETA AMINO ACID SPACER) อ.ที่ปรึกษา : รศ. ดร. ชีรยุทธ วิไลวัลย์; 390 หน้า.

งานวิจัยนี้เป็นการสังเคราะห์เพปไทด์นิวคลีอิกแอซิด (พีเอ็นเอ) ชนิดใหม่ที่มีสะพานเชื่อมเป็นกรดบีตาเอมิโน พีเอ็นเอที่สังเคราะห์ขึ้นเป็นอนุพันธ์ของพิโรลิดีนที่มีนิวคลีโอเบสทั้ง 4 ชนิดต่ออยู่ที่ตำแหน่ง C-4 โดยเฉพาะไทมีนมอนอเมอร์ได้สังเคราะห์ทั้ง 4 สเตอริโอไอโซเมอร์ ได้แก่ ซีส-ดีทรานส์-ดี, ซีส-แอล และทรานส์-แอลไอโซเมอร์ ส่วนเบสอะดีนีน, ไซโตซีน และกวานีน นั้นสังเคราะห์เฉพาะซีส-ดีไอโซเมอร์เท่านั้น ได้เชื่อมอนุพันธ์ของพิโรลิดีนเหล่านี้ด้วยกรดบีตาเอมิโนด้วยเทคนิคการสังเคราะห์เพปไทด์บนวัฏภาคของแข็ง สะพานเชื่อมในการวิจัยนี้ แบ่งได้เป็น 3 ชนิด ได้แก่ ชนิดไม่เป็นวงแหวน ประกอบด้วย เอ็น-อะมิโน เอ็น-เมทิลไกลซีน และ อะมิโนออกซีแอซิดิกแอซิด ชนิดวงแหวนที่มีไครัล 1 ตำแหน่ง ได้แก่ ดี-อะมิโน พิโรลิดีน คาร์บอกซิลิกแอซิด (ดี-เอพีซี) และแอล-อะมิโน พิโรลิดีน คาร์บอกซิลิกแอซิด (แอล-เอพีซี) และชนิดวงแหวนที่มีไครัล 2 ตำแหน่ง ได้แก่ (1S,2S), (1R,2R), (1S,2R) และ (1R,2S)-2-อะมิโนไซโคลเพนเทนคาร์บอกซิลิกแอซิด (เอซีพีซี) การสังเคราะห์ดังกล่าวมีค่าเฉลี่ยการก่อกวนในแต่ละขั้นมากกว่าร้อยละ 90 โดยได้สังเคราะห์พีเอ็นเอที่มีความยาวตั้งแต่ 5 ถึง 10 เบส และยืนยันด้วยมาลดี-ทอพ แมสสเปกโตรเมทรี พีเอ็นเอที่สังเคราะห์ได้ นำไปศึกษาการเกิดสารเชิงซ้อนระหว่างพีเอ็นเอกับดีเอ็นเอด้วยเทคนิคยูวี-ไทเทรต, การหาค่าอุณหภูมิหลอมเหลว, เจล อิเล็กโตรโฟเรซิส, เซอร์คิวลาร์ ไดโครอิซึม และฟลูออริเมทรี พบว่าพีเอ็นเอที่มีดี-เอพีซี และ (1S,2S)-เอซีพีซีเป็นตัวเชื่อม แสดงการเกิดสารเชิงซ้อนที่เสถียรกับดีเอ็นเอในอัตราส่วน 1:1 ซึ่งเป็นไปตามกฎการเข้าคู่เบสแบบวัตสัน-คริก (A·T, C·G) และพีเอ็นเอที่มีดี-เอพีซีเป็นสะพานเชื่อม มีทิศทางการเกิดสารเชิงซ้อนกับดีเอ็นเอแบบแอนติพาราเรล

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....จตุรงค์ สุภาพพร้อม.....
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CHATURONG SUPARPPROM : SYNTHESIS AND NUCLEIC ACID BINDING PROPERTIES OF NOVEL PEPTIDE NUCLEIC ACIDS CARRYING BETA AMINO ACID SPACER. THESIS ADVISOR : ASSOC. PROF. TIRAYUT VILAIVAN, D.Phil. 390 pp.

A synthetic method for novel Peptide Nucleic Acid (PNA) carrying β -amino acid spacers has been developed. The PNA monomers in this research were pyrrolidine derivatives modified with 4 natural nucleobases at C-4 position. The thymine monomers were synthesized in all possible stereoisomers including “*cis*-D”, “*trans*-D”, “*cis*-L” and “*trans*-L”. The adenine, cytosine and guanine monomers were synthesized only in “*cis*-D” configuration. These PNA monomers were coupled with β -amino acid spacers in an alternate fashion by Fmoc solid phase peptide synthesis. The spacers included in this studies were classified into three categories: acyclic (amino-oxy acetic acid, *N*-amino *N*-methylglycine), cyclic with one stereogenic center (D-aminopyrrolidine carboxylic acid or D-APC, L-aminopyrrolidine carboxylic acid or L-APC), cyclic with two stereogenic centers [(1*S*,2*S*), (1*R*,2*R*), (1*S*,2*R*) and (1*R*,2*S*)-2-aminocyclopentane carboxylic acids (ACPC)]. The solid-phase synthesis proceeded efficiently with more than 90% average efficiency for each individual coupling step. PNA sequences in 5-10 base lengths were successfully synthesized and were characterized by MALDI-TOF MS. Binding properties of these PNA with DNA were studied by UV-titration, T_m , gel electrophoresis, circular dichroism and fluorescence experiments. These revealed that the *cis*-D pyrrolidine PNA containing the D-aminopyrrolidine carboxylic acid (D-APC) or (1*S*,2*S*)-2-aminocyclopentane carboxylic acid [(1*S*,2*S*)-ACPC] spacers can form stable 1:1 hybrids with DNA. The PNA·DNA interactions followed the Watson-Crick base-pairing rules (A·T, C·G). The *cis*-D/D-APC PNA also exhibited a strong preference for binding to DNA in antiparallel fashion.

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 Field of study..... Chemistry..... Advisor's signature..... *T. Vilaiwan*
 Academic year..... 2006.....

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LIST OF ABBREVIATIONS

δ	chemical shift
μL	microliter
μmol	micromole
$[\alpha]_{\text{D}}$	specific rotation
A	adenine
A ^{Bz}	N ⁶ -benzoyladenine
Ac	acetyl
AcCl	acetyl chloride
Ac ₂ O	acetic anhydride
AcOH	acetic acid
aq	aqueous
Boc	<i>tert</i> -butoxycarbonyl
br	broad
Bz	benzoyl
c	concentration
C	cytosine
calcd	calculated
C ^{Bz}	N ⁴ -benzoylcytosine
CCA	α -cyano-4-hydroxy cinnamic acid
CDCl ₃	deuterated chloroform
d	doublet
D ₂ O	deuterium oxide
DCC	dicyclohexyl carbodiimide
DCM	dichloromethane
DCU	dicyclohexylurea
DIAD	diisopropylazodicarboxylate
DIC	diisopropyl carbodiimide
DIEA	N,N'-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	N,N'-dimethylformamide
DMSO- <i>d</i> ₆	deuterated dimethylsulfoxide

DNA	deoxyribonucleic acid
Dpm	diphenylmethyl
EDC·HCl	<i>N</i> -(3-dimethylaminopropyl)- <i>N'</i> -ethyl-carbodiimide hydrochloride
equiv	equivalent (s)
FAB ⁺	positive ion fast atom bombardment (mass spectrometry)
Fmoc	9-fluorenylmethoxycarbonyl
FmocCl	9-fluorenylmethyl chloroformate
FmocOSu	9-fluorenylsuccinimidyl carbonate
FRET	fluorescence resonance energy transfer
g	gram
G	guanine
G ^{Ibu}	<i>N</i> ² -isobutyrylguanine
G ^{Ibu} (ONpe)	<i>N</i> ² -Isobutyryl- <i>O</i> ⁷ -(<i>p</i> -nitroethyl)guanine
h	hour
HATU	<i>O</i> -(7-azabenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
HBTU	<i>O</i> -(benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
Ibu	isobutyryl
<i>J</i>	coupling constant
Lys	lysine
m	multiplet
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
MeCN	acetonitrile
MeOH	methanol
MeOTs	methyl tosylate
mg	milligram
MHz	megahertz
min	minute
mL	milliliter
mM	millimolar

mmol	millimole
mp	melting point
mRNA	messenger ribonucleic acid
MS	mass spectrometry
nm	nanometer
NMR	nuclear magnetic resonance
Npe	2-(4-nitrophenyl)ethyl
°C	degree celcius
OD _{xxx}	optical density at xxx nm
Pfp	pentafluorophenyl
PfpOH	pentafluorophenol
PfpOTfa	pentafluorophenyl trifluoroacetate
PG	an unspecified protecting group
Ph	phenyl
PNA	peptide nucleic acid or polyamide nucleic acid
ppm	part per million
PTC	phase transfer catalyst
R _f	retention factor
RNA	ribonucleic acid
rt	room temperature
s	singlet
t	triplet
T	thymine
T ^{Bz}	N ³ -benzoylthymine
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
T _m	melting temperature
t _R	retention time
Ts	<i>p</i> -toluenesulfonyl (=tosyl)
UV	ultraviolet
X	an unspecified leaving group