

การสังเคราะห์เพปไทด์นิวคลีอิกแอซิดที่มีหมู่อะมิโนเอทิลเป็นตัวเชื่อม



นางสาวพัชรี งามวิริยะวงศ์

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SYNTHESIS OF PEPTIDE NUCLEIC ACID CONTAINING  
AMINOETHYL LINKERS



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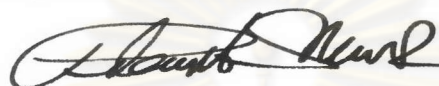
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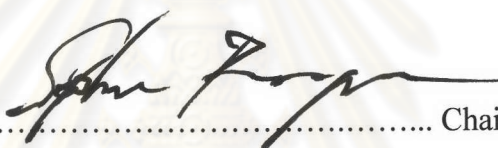
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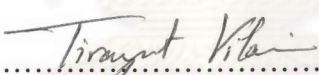
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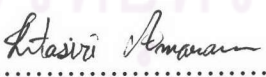
  
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งานวิจัยนี้เป็นการพัฒนาวิธีการสังเคราะห์โมโนเมอร์เปปไทด์นิวคลีอิกแอซิด (พีเอ็นเอ) ที่มีหมู่อะมิโนเอทิลเป็นตัวเชื่อม เพื่อใช้ในการสังเคราะห์พีเอ็นเอที่เป็นสารเลียนแบบดีเอ็นเอ โดยแทนที่คี่ออกซิไรโบสฟอสเฟตของดีเอ็นเอด้วยหน่วยที่คล้ายโคเปปไทด์ของคี่ออกซิไกลซิลโพรตีนวงโพรตีนมีนิวคลีโอเบส (อะดีนีน ไทมีน ไซโตซีน และ กวานีน) ต่ออยู่ที่ตำแหน่ง 4 ในลักษณะซิส และ ทรานส์ กับหมู่อะมิโนเอทิล เกิดเป็นสเตอริโอเคมี 4 แบบ คือ “ซิส-ดี” หรือ 2R, 4R “ซิส-แอล” หรือ 2S, 4S “ทรานส์-ดี” หรือ 2R, 4S “ทรานส์-แอล” หรือ 2S, 4R บนวงโพรตีน ปฏิกริยาที่สำคัญ คือ ปฏิกริยาการเชื่อมต่อกันของอนุพันธ์ของวงโพรตีน กับอะมิโนอะเซทาลดีไฮด์ โดยใช้ปฏิกริยารีดักทีฟ แอลคิลเลชัน ซึ่งปฏิกริยานี้ไม่ยุ่งยาก และให้ผลิตภัณฑ์ดีกว่าวิธีการเดิมที่เคยรายงานมา การโกลิเมอไรซ์ให้พีเอ็นเอมีความยาว 10 หน่วย ทำได้โดยการสังเคราะห์บนวัฏภาคของแข็ง ใช้การปกป้องแบบ Fmoc และการกระตุ้นด้วยเพนตะฟลูออโรพีนีลเอสเทอร์ นอกจากนี้ยังได้ศึกษาเสถียรภาพในการจับยึดเบื้องต้นระหว่างโกลิเมอไรซ์พีเอ็นเอที่สังเคราะห์ขึ้นกับโพลีนิวคลีอิกแอซิด ผลการทดลองแสดงให้เห็นว่า โกลิเมอไรซ์พีเอ็นเอที่มีความยาว 10 หน่วย ของ “ซิส” ไอโซเมอร์ สามารถจับกับโพลีอะดีนีนิก แอซิด (ตัวแทนของอาร์เอ็นเอ) ได้ดี ในขณะที่ไม่จับกับโพลีคี่ออกซิอะดีนีนิก แอซิด (ตัวแทนของดีเอ็นเอ) ในทางตรงกันข้าม “ทรานส์” ไอโซเมอร์ ไม่สามารถจับกับโพลีอะดีนีนิก แอซิด แต่จับกับโพลีคี่ออกซิอะดีนีนิก แอซิด

ศูนย์วิทยทรัพยากร  
 จุฬาลงกรณ์มหาวิทยาลัย

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A general synthetic method for monomers of Peptide Nucleic Acid (PNA) containing aminoethyl linkers has been developed. These monomers will be used for the synthesis of a PNA system whereby the entire deoxyribose phosphate was replaced by a deoxyglycyl proline dipeptide unit. The proline ring is modified by nucleobases (adenine, thymine, cytosine and guanine) at C-4 position in a *cis*- and *trans*-relationship to the carbonyl group which afforded from “*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*) absolute stereochemistry on the proline ring. The key reaction is the coupling of proline derivatives and aminoacetaldehyde by reductive alkylation. The reaction condition is simple and provides higher yields than the previously reported methods. Oligomerization of the PNAs up to 10-mers was achieved by solid phase peptide synthesis methodology employing Fmoc fragment coupling strategy and pentafluorophenyl ester activation. Preliminary binding stability of these oligoPNA with nucleic acids suggested that the homothymine decamer PNAs of “*cis*” isomers binds to poly(adenylic acid) (RNA) with high affinity, whereas no binding to the corresponding poly(deoxyadenylic acid) (DNA) was observed. On the other hand, the “*trans*” isomers failed to form stable hybrid with poly(adenylic acid) but one of it formed a rather unstable PNA·DNA hybrid.

ศูนย์วิทยุทรัพยากร  
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ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

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## LIST OF ABBREVIATIONS

A	adenine
A <sup>Bz</sup>	N <sup>4</sup> -benzoyladenine
ACN	acetonitrile
aepPNA	aminoethylprolyl peptide nucleic acid
Boc	tert-Butoxycarbonyl
br	broad
Bz	benzoyl
c	concentration
C	cytosine
°C	degree celcius
calcd	calculated
C <sup>Bz</sup>	N <sup>4</sup> -benzoylcytosine
CCA	α-cyano-4-hydroxy cinnamic acid
CDCl <sub>3</sub>	deuterated chloroform
<sup>1</sup> H- <sup>1</sup> H COSY	proton-proton correlation spectroscopy
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
dt	doublet of triplet
DCM	dichloromethane
DIAD	diisopropylazodicarboxylate
DIEA	N,N'-dimethylaminopyridine
DMF	N,N'-dimethylformamide
DMSO- <i>d</i> <sub>6</sub>	deuterated dimethylsulfoxide
DNA	deoxyribonucleic acid
Dpm	diphenylmethyl
D <sub>2</sub> O	deuterium oxide
eq.	equivalent (s)
Fmoc	9-fluorenylmethoxycarbonyl
FmocCl	9-fluorenylmethylchloroformate
g	gram

G	guanine
G <sup>Ibu</sup>	<i>N</i> <sup>2</sup> -isobutyrylgaunine
HOAt	1-hydroxy-7-azabenzotriazole
HPLC	high performance liquid chromatography
h	hour
Ibu	isobutyryl
<i>J</i>	coupling constant
Lys	lysine
M	multiplet
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
mg	milligram
MHz	megahertz
min	minute
mL	milliliter
mmol	millimole
mp.	melting point
MS	mass spectrometry
nm	nanometer
NMR	nuclear magnetic resonance
Ns	nosyl of 4-nitrobenzenesulfonyl
Pfp	pentafluorophenyl
PfpOTfa	pentafluorophenyl trifluoroacetic acid
Ph	phenyl
PNA	peptide nucleic acid or polyamide nucleic acid
ppm	part per million
RNA	ribonucleic acid
s	singlet
t	triplet
td	triplet of doublet
T	thymine
T <sup>Bz</sup>	<i>N</i> <sup>3</sup> -benzoylthymine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

$T_m$	melting temperature
$T_R$	retention time
UV	ultraviolet
$\mu\text{L}$	microliter
$\mu\text{mol}$	micromole
$\delta$	chemical shift
$[\alpha]_D$	specific rotation



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