

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47344

**IMMOBILIZATION OF PEPTIED NUCLEIC ACID ON ELECTROPHILIC  
MAGNETITE NANOPARTICLE SURFACE**

**KRITSADA TANKANYA**

**A Thesis Submitted to the Graduate School of Naresuan University  
in Partial Fulfillment of the Requirements  
for the Master of Science Degree in Industrial Chemistry**

**April 2012**

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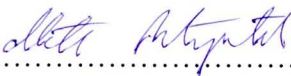
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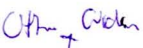



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This thesis entitled “Immobilization of peptide nucleic acid on electrophilic magnetite nanoparticle surface” submitted by Kritsada Tankanya in partial fulfillment of the requirements for the Master of Science Degree in Industrial Chemistry is hereby approved.

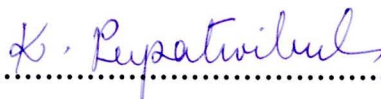
  
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### ABSTRACT

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The *aeg*PNA has become an interesting tool for biomolecular probe applications. Particularly, magnetite nanoparticles (MNPs) probe is widely investigated on its property for DNA sequencing to improve detection sensitivity. First, synthesis of Fmoc-*aeg*PNA monomers (thymine and carbazole derivatives) were prepared using HATU/2,6-lutidine and TBTU/HOBt as coupling agent (68-81% yields, respectively). Second, *ss aeg*PNA oligomers containing universal bases were synthesized manually *via* solid phase synthesis on 1  $\mu$ mole scale MBHA resin. The desired oligomer products were purified by reverse-phase HPLC. The molecular masses of target *aeg*PNA oligomers were determined by MALDI-TOF mass spectrometry. Third, determination of 2-vinyl-4,4-dimethyl-5-oxazolone (VDM) ring as electrophilic group onto MNPs loadings have been achieved by conductometric titration and it was found that the loading of active site was  $2.669 \pm 0.21 \text{ mmol.g}^{-1}$  ( $n=5$ ). Finally, desired *ss aeg*PNA oligomers were attached onto electrophilic MNPs surface monitoring by FT-IR and UV-vis spectrometry techniques. The *ss aeg*PNA oligomers coated onto novel electrophilic MNPs was prepared *via* ring-opening reaction of amino group with VDM as electrophilic group without catalyst. The 1:1 ratio between *aeg*PNA and MNPs gave the maximum immobilization. The increasing in length of *ss aeg*PNA oligomers led to a decrease of the immobilization whereas size and steric congestion at the *N*-terminus show no significant different.

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## LIST OF ABBREVIATIONS

$\delta$	=	Chemical shift
$\mu\text{M}$	=	micromolar
$\mu\text{mole}$	=	micromole
$\text{Ac}_2\text{O}$	=	acetic anhydride
$\text{AcOH}$	=	acetic acid
$\text{CDCl}_3$	=	deuterated chloroform
CBZ	=	carbazole
d	=	doublet
DCCBZ	=	3,6-dicyanocarbazole
DCM	=	dichloromethane
DIEA	=	Diisopropylethylamine
DMF	=	<i>N,N'</i> -dimethylformamide
$\text{DMSO-}d_6$	=	deuterated dimethylsulfoxide
DNA	=	deoxyribonucleic acid
DNCBZ	=	3,6-dinitrocarbazole
equiv	=	equivalent
Fmoc	=	9-fluorenylmethoxycarbonyl
g	=	gram
h	=	hour
HATU	=	<i>O</i> -(7-azabenzotriazol-1-yl)- <i>N,N,N',N''</i> -tetramethyluronium hexafluorophosphate
HOBt	=	1-Hydroxybenzotriazole
HPLC	=	high performance liquid chromatography
<i>J</i>	=	coupling constant
m	=	multiplet
MALDI-TOF	=	Matrix-assisted laser desorption/ionization-time of flight

## LIST OF ABBREVIATIONS (CONT.)

MeCN	=	acetonitrile
MeOH	=	methanol
mg	=	milligram
MHz	=	megahertz
min	=	minute
mL	=	milliliter
mM	=	millimolar
mmol	=	millimole
MS	=	mass spectrometry
$m/z$	=	mass to charge ratio
nm	=	nanometer
NMR	=	nuclear magnetic resonance
°C	=	degree Celsius
PNA	=	peptide nucleic acid or polyamide nucleic acid
ppm	=	part per million
R <sub>f</sub>	=	retention factor
s	=	singlet
t	=	triplet
T	=	thymine
TFA	=	trifluoroacetic acid
TLC	=	thin layer chromatography
UV	=	ultraviolet