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

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

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.

.....Chair
(Associate Professor Metha Rutnakornpituk, Ph.D.)

.....Committee
(Uthai Wichai, Ph.D.)

.....Committee
(Boonjira Rutnakornpituk, Ph.D.)

.....Committee
(Assistant Professor Sirirat Chancharunee, Ph.D.)

Approved

.....
(Assistant Professor Kanungnit Pupatwibul, Ph.D.)
Dean of Graduate School
30 April 2012

ACKNOWLEDGEMENT

First, I would like to sincerely thank my research advisor, Dr. Uthai Wichai, for his support, attention, patience, and guidance throughout the completion of my research. Deeply thanks are also extended to Associate Professor Dr. Metha Rutnakornpituk for electrophilic nanomaterial solid supported; Associate Professor Dr. Tirayut Vilaivan, Department of Chemistry, Faculty of Science, Chulalongkorn University for allowing me to use MALDI-TOF mass spectrometer. I also would like to thank committee members, Dr. Boonjira Rutnakronpituk, and Assistant Professor Dr. Sirirat Chancharunee for their valuable insight, comments and suggestions.

I wish to thank all staff members at Department of Chemistry, Faculty of Sciences, Naresuan University, especially Uthai's and Metha's Research Group for their assistance and friendship.

I would like to thank Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC) for tuition fee, stipend as well as research budgets.

Finally, I truly thank my parents for both of their support throughout my life and their inspiration throughout my education.

KritsadaTankanya

Title	IMMOBILIZATION OF PEPTIED NUCLEIC ACID ONTO ELECTROPHILIC MAGNETITE NANOPARTICLE SURFACE
Author	Kritsada Tankanya
Advisor	Uthai Wichai, Ph.D.
Co-Advisor	Boonjira Rutnakornpituk, Ph.D.
Academic Paper	Thesis M.S. in Industrial Chemistry, Naresuan University, 2012
Keywords	single-stranded *aeg*PNA oligomers, carbazole derivatives, universal base, electrophilic MNPs

ABSTRACT

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 μmole 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 ± 0.21 $mmol.g^{-1}$ (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.

LIST OF CONTENTS

LIST OF CONTENTS (CONT.)

LIST OF FIGURES

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF SCHEMES

LIST OF SCHEMES (CONT.)

LIST OF SCHEMES (CONT.)

LIST OF TABLES

LIST OF ABBREVIATIONS

δ	=	Chemical shift
μM	=	micromolar
μmole	=	micromole
Ac_2O	=	acetic anhydride
AcOH	=	acetic acid
$CDCl_3$	=	deuterated chloroform
CBZ	=	carbazole
d	=	doublet
DCCBZ	=	3,6-dicyanocarbazole
DCM	=	dichloromethane
DIEA	=	Diisopropylethylamine
DMF	=	*N,N'*-dimethylformamide
DMSO-d_6	=	deuterated dimethylsulfoxide
DNA	=	deoxyribonucleic acid
DNCBZ	=	3,6-dinitrocarbazole
equiv	=	equivalent
Fmoc	=	9-fluorenylmethoxycarbonyl
g	=	gram
h	=	hour
HATU	=	*O*-(7-azabenzotriazol-l-yl)-*N,N,N',N''*-tetramethyluronium hexafluorophosphate
HOBt	=	1-Hydroxybenzotriazole
HPLC	=	high performance liquid chromatography
J	=	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 resonaace
°C	=	degree celcius
PNA	=	peptide nucleic acid or polyamide nucleic acid
ppm	=	part per million
R_f	=	retention factor
s	=	singlet
t	=	triplet
T	=	thymine
TFA	=	trifluoroacetic acid
TLC	=	thin layer chromatography
UV	=	ultraviolet