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SYNTHESIS OF POLYMERS CONTAINING THYMINE

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พอลิเมอร์ชนิดใหม่ที่มีไทมีนอยู่ในทุกหน่วยซ้ำถูกสังเคราะห์ขึ้นจาก *เอ็น*^{1}-ไกลซิดิล-*เอ็น*^{3}-เบนโซอิลไทมีน (**XIV**) โดยปฏิกิริยาพอลิเมอไรเซชันเปิดวงแบบแคตไอออนิก ซึ่งมีโบรอนไตรฟลูออไรด์เป็นตัวริเริ่มปฏิกิริยา โดยสังเคราะห์มอนอเมอร์ (**XIV**) จาก *เอ็น*^{3}-เบนโซอิลไทมีน (**XII**) ที่ทำปฏิกิริยากับไกลซิดอล (**XIII**) ด้วยปฏิกิริยามิทสึโนบุ แต่พบว่าหมู่เบนโซอิลซึ่งเป็นหมู่ปกป้องถูกกำจัดออกในระหว่างการเกิดปฏิกิริยาพอลิเมอไรเซชัน การกำจัดหมู่เบนโซอิลทั้งหมดทำได้โดยใช้ aq.NH_3:MeOH ในอัตราส่วน 1:1 การวิเคราะห์โครงสร้างของพอลิ(*เอ็น*^{1}-ไกลซิดิลไทมีน) (**XV**) ทำโดยเอ็นเอ็มอาร์และเอฟที-ไออาร์ น้ำหนักโมเลกุลเฉลี่ยที่วัดได้ประมาณ 600 ดาลตัน น้ำหนักโมเลกุลสูงสุดของผลิตภัณฑ์ที่วัดได้คือ 1,670 ดาลตัน ผลิตภัณฑ์ที่สังเคราะห์ได้มีน้ำหนักโมเลกุลต่ำเนื่องจากตัวริเริ่มสามารถจับกับหมู่คาร์บอนิลในมอนอเมอร์ แล้วทำให้ความว่องไวลดลง อุณหภูมิสลายตัวของไทมีนโอลิโกเมอร์วัดโดยทีจีเอได้ 245 องศาเซลเซียส และอุณหภูมิที่สารเริ่มเปลี่ยนจากสถานะคล้ายแก้วไปเป็นสถานะคล้ายยาง (T_g) คือ 36 องศาเซลเซียส ลักษณะการดูดกลืนแสงยูวีของไทมีนโอลิโกเมอร์คล้ายกับไทมีนมากกว่า *เอ็น*^{1}-ไกลซิดิล-*เอ็น*^{3}-เบนโซอิลไทมีน แสดงว่ามีไทมีนอิสระอยู่ในผลิตภัณฑ์ การศึกษาการจับกันของโมเลกุลผลิตภัณฑ์ที่ได้กับพอลิ(ดีออกซีอะดินิลิกแอซิด)และพอลิ(อะดินิลิกแอซิด)พบว่าไม่มีการจับตัวกันระหว่างไทมีนโอลิโกเมอร์กับพอลินิวคลีโอไทด์ทั้งสองแบบ

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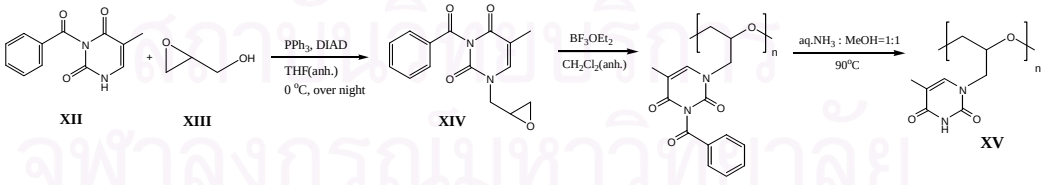

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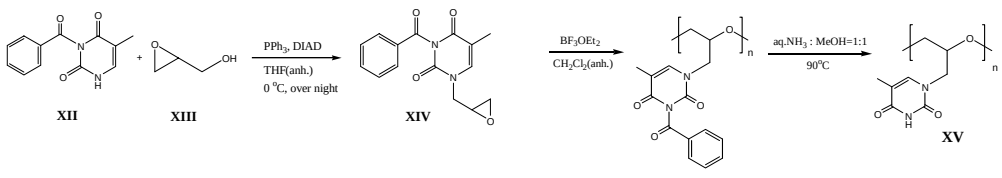
KEY WORD: THYMINE/ CATIONIC RING-OPENING POLYMERIZATION/ BF_3

KARNJANA TANAPAIBOON: SYNTHESIS OF POLYMER CONTAINING THYMINE. THESIS ADVISOR: ASST. PROF. VARAWUT TANGPASUTHADOL, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. TIRAYUT VILAIVAN, D.Phil., 71 pp. ISBN 974-17-6590-8

Novel oligomers with thymine in every repeat unit was synthesized using cationic ring opening polymerization of N^1-glycidyl-N^3-benzoylthymine (**XIV**) in the presence of BF_3 as an initiator. The monomer (**XIV**) was synthesized from the reaction of N^3-benzoylthymine (**XII**) with glycidol (**XIII**) by Mitsunobu reaction. During the polymerization some benzoyl protecting groups were removed. Complete removal of the benzoyl protecting group was carried out by using aq.NH_3:MeOH (1:1). The structure of poly(N^1-glycidylthymine) (**XV**) was verified by NMR and FT-IR. The weight-average molecular weight of about 600 Da was achieved. The maximum detectable molecular weight was 1,670 Da. The low molecular weight product was obtained because the initiator was most likely deactivated after binding to the carbonyl groups in the monomer. The decomposition temperature, measured by TGA, was obtained at 245°C. The glass transition temperature was found at 36°C. The UV absorbance of the product is similar to thymine more than that of N^1-glycidyl-N^3-benzoylthymine, indicating that the obtained product contained thymine unit. Its interactions with poly(deoxyadenylic acid) and poly(adenylic acid) were, however, not detected.

Synthetic scheme



XII **XIII** **XIV** **XV**

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LIST OF ABBREVIATIONS

$\overline{M}_w$	weight-average molecular weight
$\overline{M}_n$	number-average molecular weight
°C	degree celsius
AROP	anionic ring-opening polymerization
$BF_3 \cdot Et_2O$	Boron trifluoride etherate
br	broad
BzCl	benzoyl chloride
$CDCl_3$	deuterated chloroform
δ	chemical shift
CROP	cationic ring-opening polymerization
d	doublet
dA_{50}	fifty units of deoxyadenylic acid
dd	doublet of doublet
DIAD	diisopropyl azodicaboxylate
DMSO-d_6	deuterated dimethylsulfoxide
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
eq	equivalent
EtOAc	ethyl acetate
g	gram
h	hour
Hz	hertz
J	coupling constant
m	multiplet
m/z	mass per charge

m-CPBA	*meta*-chloroperbenzoic acid
MeOH	methanol
mg	milligram
min	minute
mL	milliliter
MS	mass spectrometry
Na_2SO_4	Sodium sulphate
$NaHCO_3$	Sodium hydrogen carbonate
NMR	nuclear magnetic resonance
PDI	polydispersity index
Ph	phenyl
Poly(dA)	poly(deoxyadenylic acid)
Poly(rA)	poly(adenylic acid)
PPh_3	triphenylphosphine
ppm	part per million
q	quartet
rA_{50}	fifty units of adenylic acid
rt	room temperature
s	singlet
$SnCl_4$	Stannic chloride
T_d	decomposition temperature
T_g	glass transition temperature
TGA	thermogravimetric Analysis
THF	tetrahydrofuran
TLC	thin layer chromatography
T_m	melting temperature
UV	ultraviolet spectroscopy

CHAPTER I

INTRODUCTION

Nowadays, self assembly of synthetic molecules into two- or three-dimensionality has received considerable attention from researchers in various disciplines. Special attention has been paid to the design and synthesis of molecules which are capable of forming an assembly via specific interaction with another molecule specifically. This kind of assembly occurs naturally. Examples include an interaction between different polypeptide chains via hydrogen bonding, and an interaction between polynucleotide chains forming double helix structure of DNA. Note that the latter structure is more specific than the former.

Molecular recognition of base pair in the structure of nucleotide can be described by Watson-Cricks rule [1], in which adenine (A) forming an adduct with thymine and uracil (T and U, respectively), whereas cytosine (C) forming an adduct with guanine (G). Structures of these nucleobases pairing are shown in Figure 1.1.

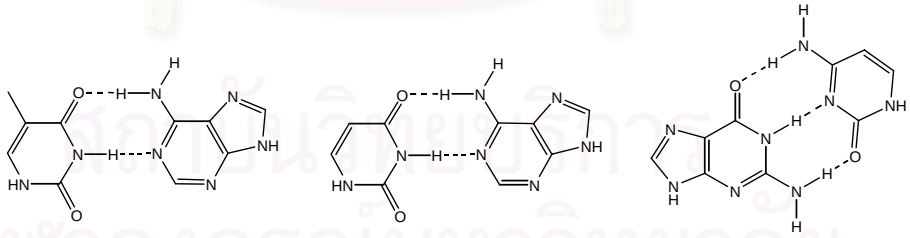


Figure 1.1 Watson-Cricks Pairing rules for nucleobases.

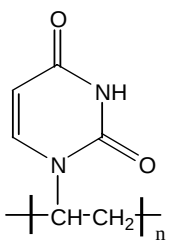
The special feature as mentioned above stimulates various researchers to synthesize polymers structure of which are similar to the structure of polynucleotides. By this way a model of naturally-occurring polynucleotides can be made. Assumption in doing this is that the synthesized polymers may exhibit some specific properties

such as base-stacking, self assembly, or more importantly, binding properties of genetic materials such as DNA or RNA. This will lead to a solution for some diseases according to the anti-sense principle [2]. However, utilization of natural polynucleotides often encounters problems such as the degradability of phosphate bond by hydrolysis, or by the nuclease enzymes contained in living organisms. Therefore, this research will focus on the synthesis of polynucleotides containing polymer chains which are not degraded easily, but possessing a nucleobase part which is similar to a polynucleotide obtained naturally.

1.1 Theory

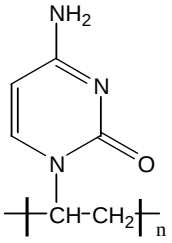
1.1.1 Design of nucleobase recognition for biotechnology

The polynucleotide chain exhibits a remarkable property, namely the specific recognition between two polynucleotide chains to form the helix structure of DNA, hence enabling the transfer of genetic characters. This makes the model research on polynucleotide analogues an interesting area. Scientists are interested in the preparation and modeling of synthetic polymers having similar features to those of natural polynucleotide. It is assumed that a synthetic polymer possessing nucleotide bases should, similar to natural nucleotides, exhibit intramolecular base-stacking or intermolecular self assembly base-pairing as well.



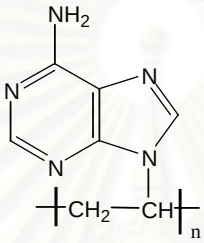
Poly(1-vinyluracil)

[poly VU]



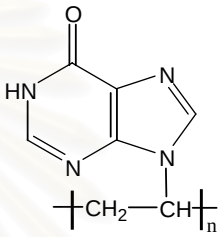
Poly(1-vinylcytosine)

[poly VC]



Poly(9-vinyladenine)

[poly VA]



Poly(9-vinylhypoxanthine)

[poly VHX]

Scheme 1.1 Four vinyl polymers

In order to better understand the relationship between the structure and function of nucleic acids, many analogs and model systems of nucleic acids have been devised and studied. Nucleic acid analogs with the sugar-phosphate backbone replaced by a backbone containing only carbon-carbon linkages have been prepared. Josef Pitha *et al.* [3]. have studied a set of four vinyl polymers, where the base is uracil, cytosine, adenine, or hypoxanthine (Scheme 1.1).

The antiviral activities of analogs of the double-stranded complex of polyinosinic and poylcytidylic acids [poly(I)·poly(C)], which is a potent interferon inducer, have been studied by the same researcher [4]. Structural changes that modify the polymer backbone substantially, such as loops or $2' \rightarrow 5'$ phosphodiester bonds, lead to decreased antiviral activity. Unexpectedly, however, the complex of polyinosinic acid and poly(1-vinylcytosine), which is only a much more distantly related analog of poly(I)·poly(C), shows high activity. It is postulated that the high

activity is related to the reduction of the charge/mass ratio and to the existence of this complex in an aggregated state.

These vinyl polymers differ from polynucleotides by the absence of negatively charged phosphate groups, shorter distances between the bases, and the lack of steric regularity. These differences exclude complete base pairing in any complex of a vinyl polymer with a polynucleotide [5].

Synthesis of polymer similar to polynucleotide was reported before by modification of phosphates and riboses into other functional groups. For example, Marsh *et al*. [6]. synthesized poly(methyl methacrylate) and polystyrene terminally functionalized with 5'-methacryloyluridine and 5'-methacryloyladenine as monomers. These monomers had been polymerized under atom transfer polymerization conditions, giving products of narrow polydispersities. Furthermore, polymerization could occur by using both adenosine and uridine derived initiators.

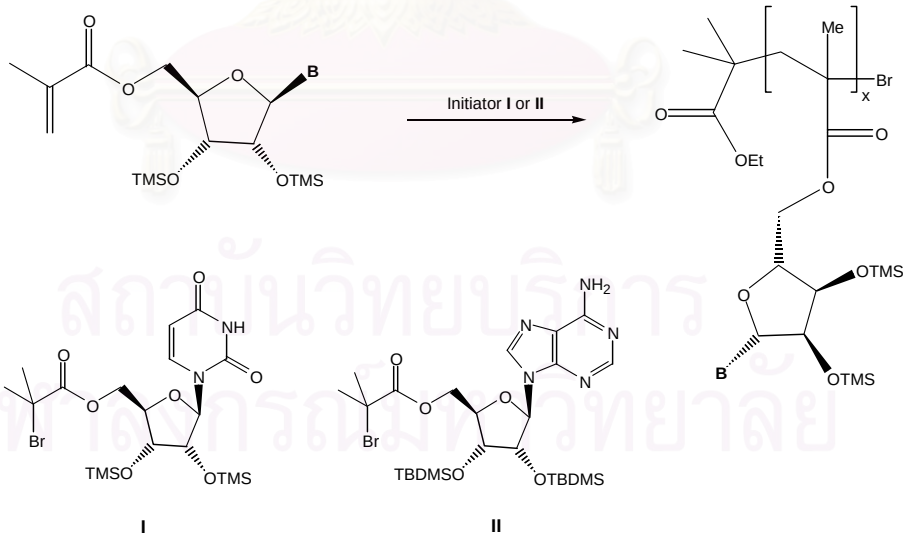


Figure 1.2 Polymerization of 5'-methacryloyluridine or 5'-methacryloyladenine in the presence of uridine or adenosine derived initiators (**I**, **II**), (B = uracil, adenine) [6]

The same research group also reported that poly(5'-acryloyluridine) was able to act as a template in the radical polymerization of the complementary 5'-acryloyladenosine in the presence of the noncomplementary 5'-acryloyluridine (Figure 1.3-1.4) [7].

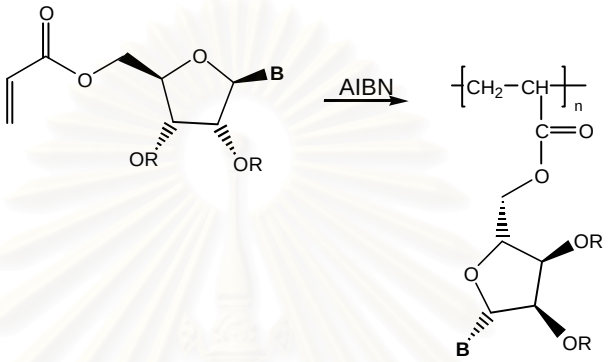


Figure 1.3 The radical polymerization of the 5'-acryloylnucleobase. (**B** is adenosine or uridine)

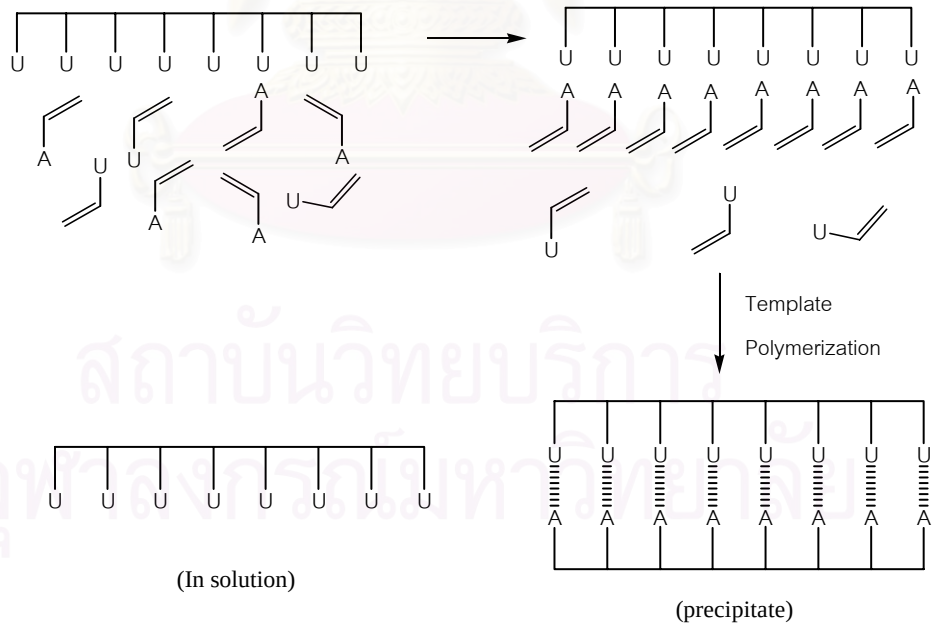


Figure 1.4 Use of poly(5'-acryloyluridine) as a template in the synthesis of poly(5'-acryloyladenosine) (A is adenosine, U is uridine)

Han *et al*. [8] reported the alternating cyclo-copolymerization between nucleoside derivatives and acrylic anhydride or maleic anhydride. The obtained copolymer formed the base-paired complexes with the natural polymer of complementary bases, which were confirmed by UV absorption spectroscopy.

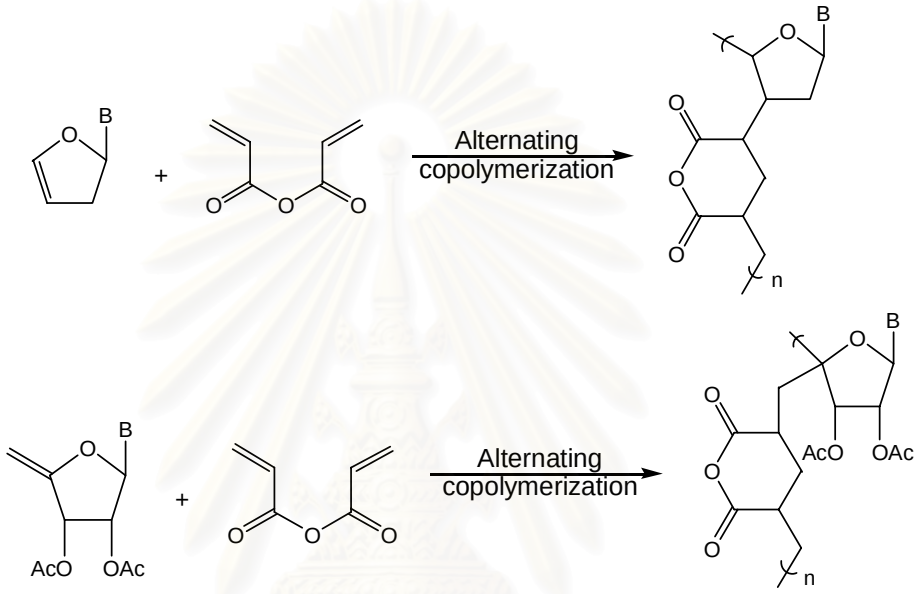


Figure 1.5 synthesis of alternating copolymer between nucleotide and acrylic anhydride [8]

All of the polynucleotide analogues that have been reported so far are the analogues to polydeoxyribonucleotide. Later, polyribonucleotide (RNA) analogues (Figure1.6) containing hydroxyl groups on C-2′ of furanose rings were prepared. These polyribonucleotide analogues were carried out in bulk in the presence of radical initiator (AIBN) [9].

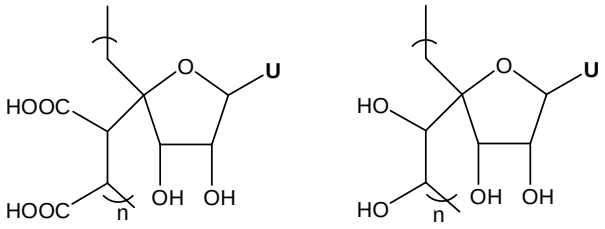


Figure 1.6 The polyribonucleotide (RNA) analogues (**U** is uridine).

Lowe and Vilaivan [10, 11] reported the synthesis of peptide nucleic acid (PNA), where the sugar phosphate backbone of oligonucleotide analogues was replaced by a peptide chain. They reported the design and synthesis of amino acid bearing nucleobases with a view to their use for the synthesis of novel peptide nucleic acids.

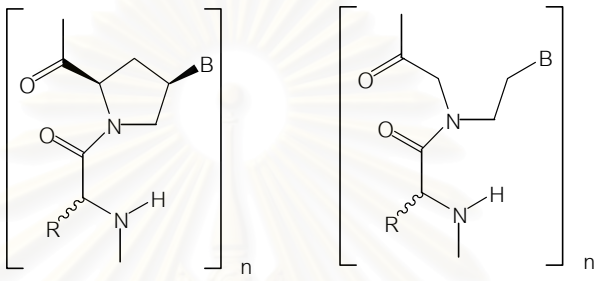


Figure 1.7 Examples polymer containing nucleobase (B = nucleobase) [10, 11]

The modification of macromolecule based on the advantages of hydrogen bonding was reported by Koji Yamauchi *et al.* [12]. The synthesis and characterization of novel multiple hydrogen bonding (MHB)-terminated telechelic polyesters was reported (Figure 1.8). It was demonstrated that the advantages of MHB containing macromolecules was to improve of mechanical properties and flow characteristics during melt processing.

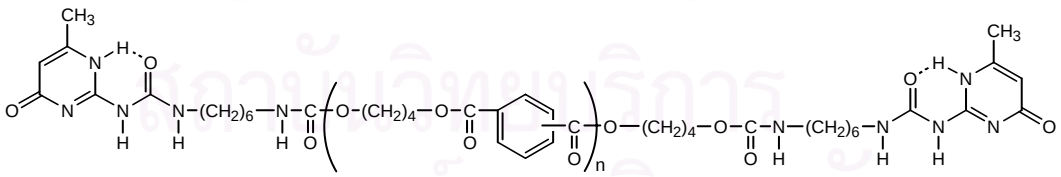


Figure 1.8 The MHB-terminated telechelic polyester.

Beside, Stubbs *et al.* [13] reported new polymers containing terminal hydrogen-bonding recognition motifs, based on diaminotriazine and diaminopyridine groups in their side chains for the self-assembly of appropriate receptors. The polymer was prepared by ring-opening metathesis polymerization (ROMP) of norbornenes.

They exhibit a high affinity for hydrogen-bonded receptors on both monomeric and polymeric level.

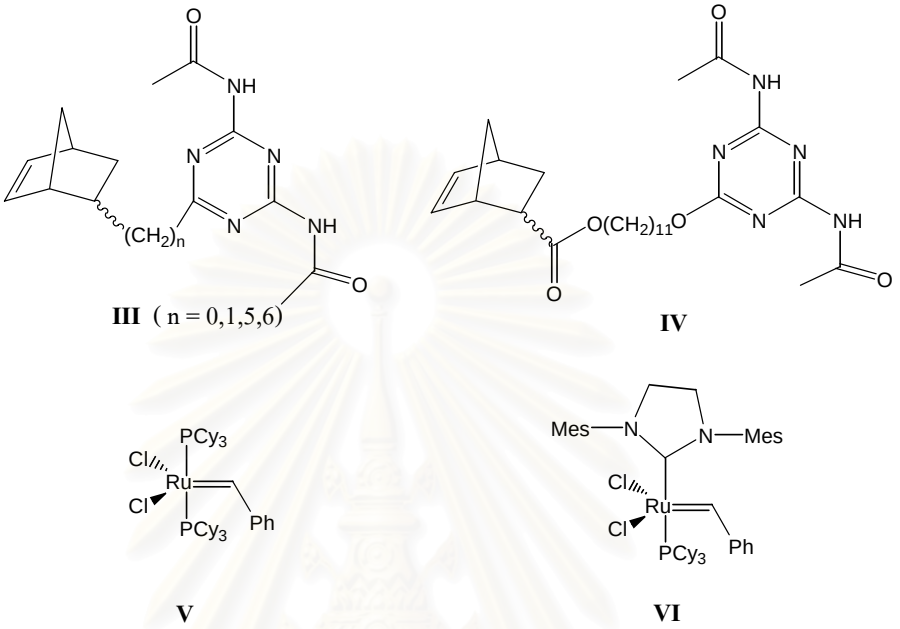


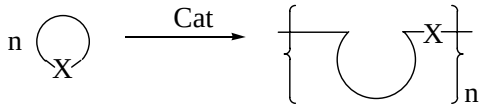
Figure 1.9 The norbonene monomers base on diaminotriazine (**III**) and diaminopyridine (**IV**) were polymerized by using ruthenium catalyst (**V**, **VI**)

The above-mentioned examples are the synthesis of polymer containing nucleobases. These products exhibit molecular recognition between complementary base similar to DNA or RNA. In this study, the goal polymers are synthesized via ring opening polymerization of an epoxide monomer containing a nucleobase, thymine.

1.1.2 Ring-Opening Polymerization [14]

Ring-opening polymerization (ROP) constitutes one of the most important fields of polymer chemistry. Along with step and chain mechanisms for the formation of polymers, ring-opening reaction provides an important methodology for polymer formation (Scheme 1.2). In ROP, no small molecule by-products are formed during polymerization as in condensation polymerization. It also does not involve the

exothermic driving force of conversion of multiple bonds to single bonds, as in olefin polymerization.



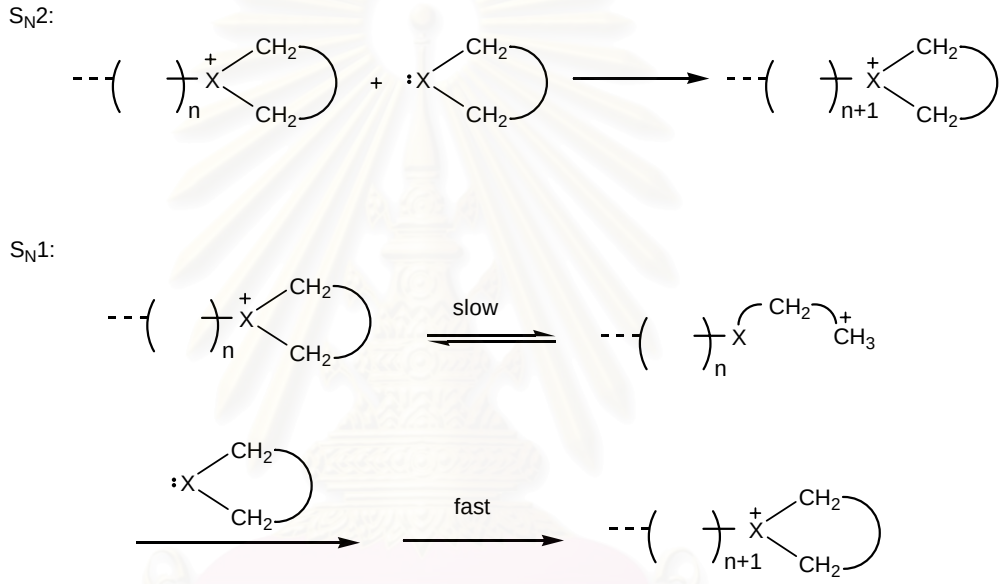
Scheme 1.2 Ring-opening polymerization (X is heteroatom such as oxygen).

The growth process in ROP has some of the characteristic of chain polymerization. Only monomer adds to the growing chains in a propagation step. Species larger than monomer do not react with each other. The classification of a ring-opening polymerization as a chain or step polymerization can be made on the basis of two criteria: (1) the experimentally observed kinetic laws and (2) the relationship between polymer molecular weight and conversion. The second criterion is the prime characteristic that distinguishes chain and step polymerizations. High polymer is formed throughout the course of a chain polymerization in contrast to the slow building of polymer molecular weight in step polymerization. Most, but not all, ROPs behave as step polymerizations in that the polymer molecular weight increases relatively slowly with conversion. This is because the rate constants for ring-opening polymerization of cyclic monomers, such as ethers, and esters, have values much closer to those for the reactions of step polymerization (*e.g.*, esterification, amidation) than for chain polymerization (addition of radical, carbocation, or carbanion to C=C).

In many cases, ring-opening polymerization by a “living” mechanism is possible. That is, the initiation step is sufficiently faster than the propagation step, such that each molecule of initiator becomes associated with a growing chain of polymer. In addition, propagation steps are required to be faster than termination reactions, so that chains continue to grow until all the monomer is depleted. The ROP reaction can be achieved either by anionic or cationic processes.

Cationic Ring-opening Polymerization (CROP)

In CROP the propagation reaction can be described as a nucleophilic reaction, in which the positively charged active species is the electrophile and the monomer is the nucleophile. Their interaction can be classified as the S_N2 type or as the S_N1 type process (Scheme 1.3).



Scheme 1.3 S_N1 and S_N2 mechanism in propagation step of CROP or active chain end mechanism (ACE) (counter ion omitted; X is a heteroatom).

The S_N1 mechanism is favored if the structure of the monomer is such that the resulting carbenium ion is stabilized and if the monomer is a weak nucleophile. This is the case of, *e.g.*, some cyclic acetals and cyclic orthoesters.

In the polymerization mechanisms described above, ions are located at the end of macromolecules, thus, the process is called *active chain end polymerization* (ACE). More recently a new mechanism of propagation has been postulated, for termed *activated monomer polymerization* (AM). In this polymerization, the growing end of

the molecule is (*e.g.*, for cyclic ethers) an –OH group. The positive charge is located on the monomer molecules (Scheme 1.4).

Scheme 1.4 Activated monomer mechanism (AM) in propagation step of CROP.

In CROP, proceeding by the ACE mechanism, the bond breaking in the active chain end (onium ion) has been shown to be the decisive factor, determining the rate constant of propagation for a given monomer. It is well known that carbon-onium bonds provide better leaving groups compared with the corresponding carbon-heteroatom bonds (*e.g.*, ammonium *vs.* amine, oxonium *vs.* ether). Therefore, it is not surprising that more heterocyclic can be polymerized by a cationic mechanism than by an anionic mechanism.

For instances, Dworak *et al*. [15] reported the polymerization of glycidol under the action of various types of Lewis acids ($BF_3 \cdot Et_2O$, $SnCl_4$) and protonic acids (CF_3COOH, CF_3SO_3H) as shown in Figure 1.10. Polymer with number-average molecular weights ($\overline{M}_n$) varying from 2,500 to 6,000 g/mol was obtained.

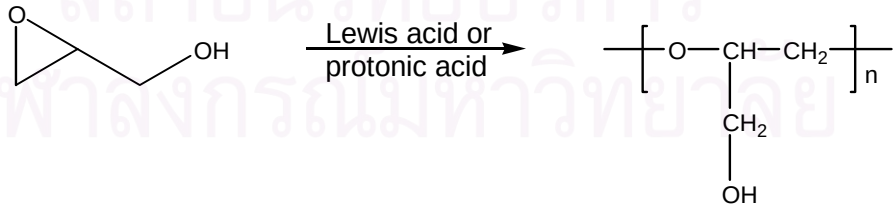


Figure 1.10 The cationic ring-opening polymerization of glycidol the presence of Lewis acid ($BF_3 \cdot Et_2O$, $SnCl_4$) or protonic acid (CF_3COOH, CF_3SO_3H).

Francis *et al*. [16] also utilized the same Lewis acid catalyst, e.g., $BF_3 \cdot Et_2O$, $SnCl_4$, as an initiator in the polymerization of epichlorohydrin in the presence of diols

as shown in Figure 1.11. The molecular weight of the polymer increased with increasing the epichlorohydrin/diol ratio in the reaction mixture.

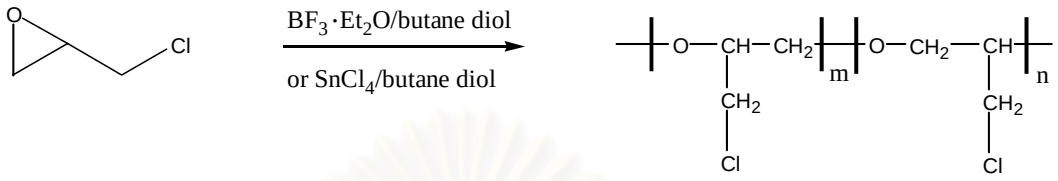


Figure 1.11 The cationic ring-opening polymerization of epichlorohydrin.

The ring-opening cationic polymerization synthesis of novel photoreflective poly(glycidyl ether) oligomers of a carbazole-based multifunctional chromophore was reported in 2000 by Sohn *et al*. [17]. The glycidyl ether monomer was polymerized in the presence of $BF_3 \cdot Et_2O$ as shown in Figure 1.12.

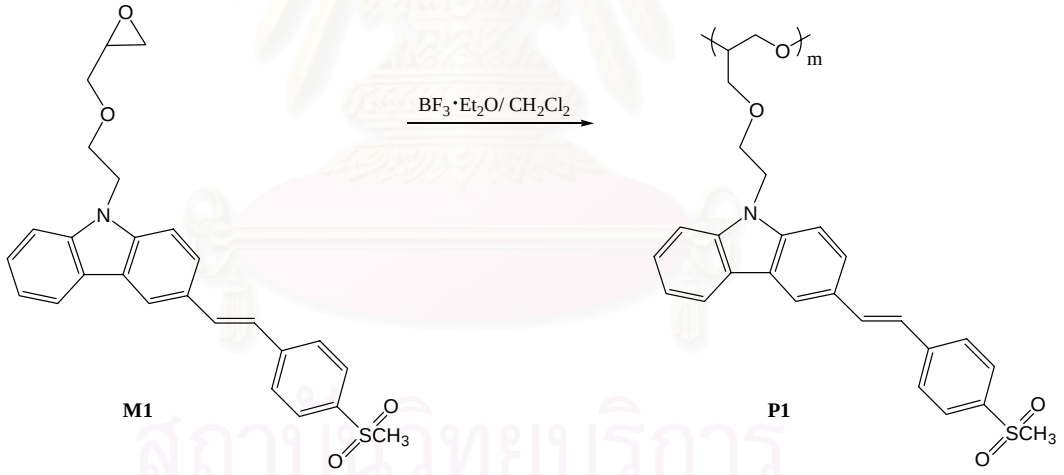


Figure 1.12 Polymerization of **M1** into **P1** using $BF_3 \cdot Et_2O$ as an initiator. The reaction was carried out at 0°C for 18 h.

For these examples, the $BF_3 \cdot Et_2O$ and $SnCl_4$ are potentially used for CROP of glycidyl derivative monomer in this study.

Anionic Ring-opening Polymerization (AROP)

Typically, in an anionic ring-opening polymerization each propagation step involves a nucleophilic attack of the anionic active center, located at the end of the growing macromolecule, on the heterocyclic monomer (Scheme 1.5). This attack results in an extension of the length of the polymer chain with regeneration of the active center at the terminal position.

X denotes heteroatom (e.g., X=O or S) or group including heteroatoms (e.g., C(O)O); cat$^+$ means the monovalent metal (e.g., Li$^+$, Na$^+$, K$^+$, Cs$^+$) or onium (e.g., R_4N^+ , R_4P^+) cations.

Scheme 1.5 Propagation step of AROP.

For polymerizations with active centers bearing multivalent metal atoms (*e.g.*, Cd, Zn, Mg, Al, and Sn), the propagation step can be written in a way similar to Scheme 1.5. However, there are indications that such active centers are not ionized, and thus, in contrast to polymerizations with alkali metal counter ions. In this system, propagation proceeds on covalent active species, called "pseudo-anionic polymerization".

In spite of the substantial progress made within the last two decades anionic ring-opening polymerization cannot be considered a closed field. Although the number of cyclic monomers known to polymerize anionically is rather limited, the anionic ring-opening polymerization often creates unique possibilities of the controlled synthesis of macromolecules with various regularly repeated carbon and heteroatoms.

1.1.3 Polymer Molecular Weight [18, 19, 20]

Molecular weight is an extreamly important variable because it relates directly to a polymer's physical properties. In general, the higher the molecular weight, the tougher the polymer; however, too high a molecular weight can lead to processing difficulties. Some physical properties include the temperatures for transitions from liquids to waxes to rubbers to solids and mechanical properties such as stiffness, strength, viscoelasticity, toughness, and viscosity. If molecular weight is too low, the transition temperatures and the mechanical properties will generally be too low for the polymer material to have any useful commercial applications. For a polymer to be useful it must have transition temperatures to waxes or liquids that are above room temperatures and it must have mechanical properties sufficient to bear design loads.

A given polymer material is mostly a mixture of molecule of (nearly) indentical chemical structure but varying in chain length or molecular weight. The molecules produced in polymerization reactions have lengths that are distributed in accordance with a probability function which is governed by the mechanism of the reaction and by the conditions under which it has been carried out. The concept of average molecular weight is therefore important and relevant and the assignment of a numerical value to the molecular weight of polymer requires the definition of a particular average. Two most important averages are: (i) number average molecular weight ($\overline{M}_n$) and (ii) weight average molecular weight ($\overline{M}_w$).

Number average molecular weight [19, 21]

The number average molecular weight is a way of determining the molecular weight of a polymer. Polymer molecules, even ones of the same type, come in different sizes (chain lengths, for linear polymers), so the average molecular weight will depend on the method of averaging. The *number average* molecular weight is the

common average of the molecular weights of the individual polymers. It is determined by measuring the total weight of polymer divided by the number of polymer molecules. This average molecular weight follows the conventional definition for the mean value of any statistical quantity. In polymer science, it is called the number average molecular weight ($\overline{M}_n$).

Weight average molecular weight [18, 19]

Consider of polymer property which depends not just on the number of polymer molecules but on the size or weight of each polymer molecule. A classic example is light scattering. The greater the mass, the greater is the contribution to the measurement.

Polydispersity index [19, 21, 22]

The polydispersity index (PDI) is a measure of the distribution of molecular weights in a given polymer sample. For any molecular weight distribution, the average molecular weights always rank in the order $\overline{M}_w \geq \overline{M}_n$ (Figure 1.13). The equality of $\overline{M}_w$ and $\overline{M}_n$ would correspond with a perfectly uniform (*monodisperse*) sample. The ratio of these average molecular weight is often used as a guide to the dispersity of the chain lengths in a polymer sample. The greater $\overline{M}_w / \overline{M}_n$ the greater is the dispersity. For most, though not all, bulk properties $\overline{M}_w$ is a more informative average than $\overline{M}_n$.

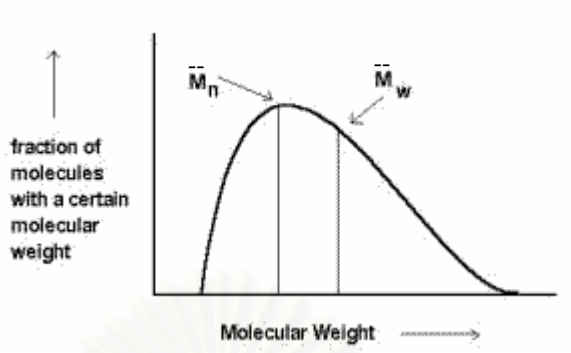


Figure 1.13 A typical differential molecular weight distribution curve [23]

Typical PDI's vary based on the mechanism of polymerization and can be affected by a variety of reaction conditions. For typical addition polymerization, values of the PDI can range around 10 to 20. For typical step polymerization, values range around 2 to 3. Living polymerization, a special case of addition polymerization, leads to values very close to 1.

1.1.4 Thermal properties [18]

Thermal properties of polymer are variably. Example are crystalline melting point, glass transition temperature, flammability, and thermal stability. Flammability and thermal stability are concerned with chemical transformations, whereas melting and glass transition temperatures represent morphological changes.

Glass Transition Temperature

One of the most important characteristics of the amorphous state is the behavior of a polymer during its transition from solid to liquid. If an amorphous glass is heated, the kinetic energy of the molecules increases. Motion is still restricted, However, to short-range vibrations and rotations so long as the polymer retains its glasslike structure. As temperature is increased further, there comes a point where a decided change takes place; the polymer losed its glasslike properties and assumes

those more commonly identified with a rubber. The temperature at which this takes place is called the *glass transition temperature (T_g)*. If heating is continued, the polymer will eventually lose its elastomeric properties and melt to a flowable liquid.

Thermal Decomposition [24]

Thermal decomposition (T_d) define as the temperature that indicated the chemical decomposition of a polymer at elevated temperatures usually becomes evidant in a practical sense by a deterioration of the physical properties. Such chemical information can often be used to predict the property changes expected when a polymer is used at high temperatures. For example, depolymerization of the polymer would be expected to result in a loss of strength, increasing brittleness, and perhaps even liquefaction. However, a more meaningful test of technological thermal stability is to examine the actual mechanical properties of the material after it has been heated ("aged") for various periods of time at elevated temperatures.

1.2 Interaction of oligonucleotide with nucleic acid [25]

As a duplex can be denatured either chemically (sodium hydroxide) or by heat a measure of the stability can be found by measuring a value known as the melting temperature, T_m. The melting temperature is defined as the temperature at which 50% of the DNA exists as a duplex and 50% is single stranded. The melting temperature of a duplex can be estimated from the base pair content of the duplex.

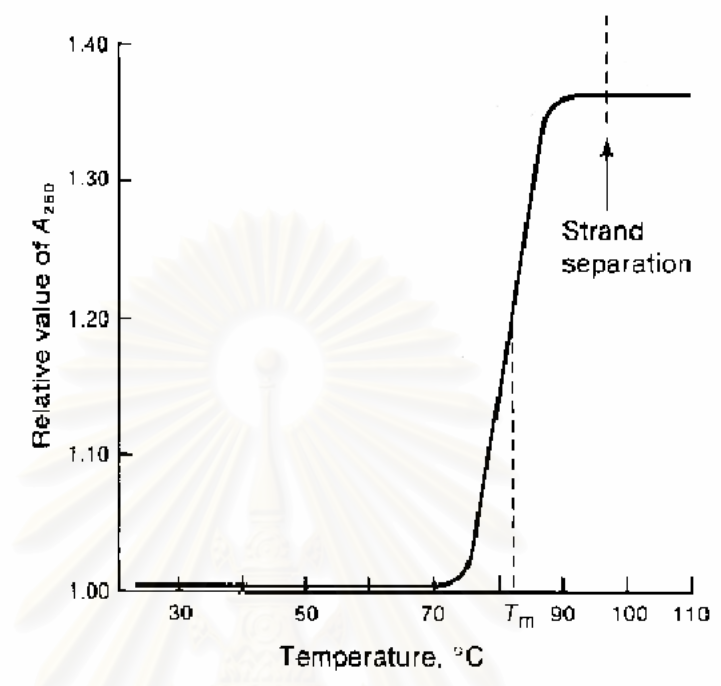


Figure 1.14 The UV absorbance of T_m experiment

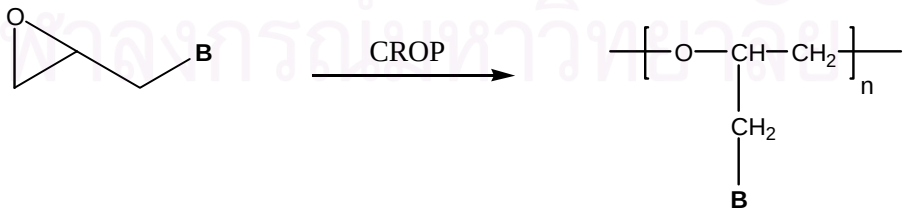
An accurate measure of the melting temperature can be obtained by conducting a UV melting experiment. The UV absorbance of single stranded DNA differs from duplex DNA and is higher. This is due to a property known as hypochromicity and arises from the coupling of transition dipoles between neighboring stacked bases. Therefore by measuring the UV absorbance as the temperature increases gives a curve as shown in Figure 1.14. By taking the first order derivative of the curve a value for the T_m can be calculated. This value is dependent upon salt concentration and pH. Another factor which alters the value of the T_m is the concentration of the DNA.

1.3 Statement of hypotheses

The above-mentioned examples are the synthesis of polymers possessing structure similar to that of RNA or DNA, i.e., C-C bond constitutes for the backbone of a polymer. This research aims at the synthesis of nucleobase-containing polymers with C-O-C backbone in the manner similar to the backbone of poly(ethylene oxide) or poly(ethylene glycol). These polymers provide special benefits for use as a medical polymer such as solubility, biocompatibility, and biodegradability. Starting from glycidyl-derived monomers, the required polymers are synthesized via cationic ring opening polymerization of an epoxide ring using a suitable initiator.

1.4 Objective of this research

The objective of this research is to synthesize a novel polymer possessing a nucleobase in every repeating unit. The backbone of a polymer chain is an ethylene oxide, structure of which is similar to poly(ethylene oxide), which are flexible and water-soluble. These types of polymer have many advantages such as solubility in water, biocompatibility and biodegradability. The monomer used is based on a glycidyl derivative containing a nucleobase. The monomer is polymerized by a cation polymerization method coupled with ring opening using a suitable initiator (Scheme 1.6).



Scheme 1.6 Polymer syntheses (**B** is nucleobase)

CHAPTER II

EXPERIMENTAL METHODS

2.1 Experimental Design

The plan of this study starts with synthesis of N^1-glycidyl-N^3-benzoylthymine. Two synthesis methods were evaluated. In the first method, N^1-glycidyl-N^3-benzoylthymine was the product from Mitsunobu reaction between N^3-benzoylthymine and glycidol. The second method is an epoxidation of N^1-allyl-N^3-benzoylthymine. Structural identification of the product was carried out by FT-IR, NMR, and elemental analysis.

The next step was the polymerization of the monomer by CROP in the presence of Lewis acid such as $BF_3 \cdot Et_2O$ and $SnCl_4$. After that, the benzoyl protecting group was removed in basic condition to obtain the final product. The structural identification of final product was verified by FT-IR, NMR, and MS.

The molecular weight determination of product was measured by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). Thermal properties were analyzed by DSC and TGA.

DNA and RNA binding capability of the final product was also determined by UV melting method.

2.2 Materials

Glycidol was distilled under reduced pressure before use. All initiators, and other chemicals were used as received without further purification. Commercial-grade solvents were distilled before use. Anhydrous dichloromethane and tetrahydrofuran was prepared by distillation under nitrogen atmosphere.

A List of Chemicals and their manufacturers.

1. Allyl alcohol	Merck
2. Ammonia Solution	BDH laboratory Supplies
3. Benzoyl chloride	Aldrich
4. Boron trifluoride etherate ($BF_3 \cdot Et_2O$)	Fluka
5. Dichloromethane	Commercial grade, Merck
6. Diethyl ether	AR grade, Merck
7. *N, N*-diisopropyl azocarbodiimide (DIAD)	Fluka
8. Dimethyl sulfoxide (DMSO)	AR grade, Merck
9. Dioxane	AR grade, Merck
10. Ethyl acetate	Commercial grade, Merck
11. Glycidol	Aldrich
12. Hexane	Commercial grade, Merck
13. Hydrochloric acid	Merck
14. *meta*-chloroperbenzoic acid (*m*-CPBA)	Fluka
15. Methanol	Commercial grade, Merck
16. Poly(dA) (dA_{50})	Bioservice Unit (BSU)
17. Poly(rA) (rA_{50})	Bioservice Unit (BSU)
18. Potassium carbonate	Fluka
19. Pyridine	Fluka
20. Sodium hydrogen carbonate ($NaHCO_3$)	Riedel-de-Haën
21. Sodium sulphate (Na_2SO_4)	Merck
22. Stannic chloride ($SnCl_4$)	Fluka
23. Tetrahydrofuran	AR grade, Merck
24. Thymine	Aldrich
25. Triphenylphosphine (PPh_3)	Fluka

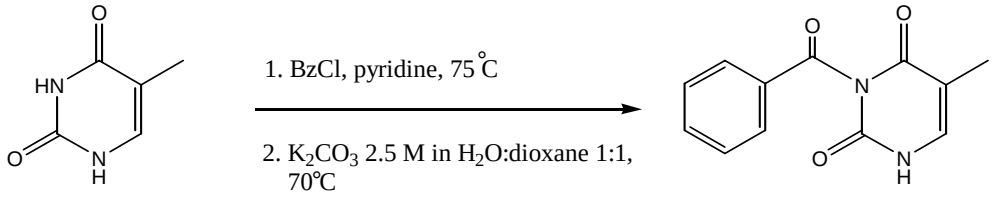
2.3 General methods for product characterization

The weights of all chemical substances were determined on a Mettler AB 204-S electrical balance. Evaporation of solvents was carried out on a Büchi Rotavapor R-200 equipped with a Büchi Heating Bath B-490. The progress of the reactions was followed by thin layer chromatography (TLC) performed on Merck silica gel 60 F_{254} precoated aluminium plates and visualized using either UV light (254nm) or iodine. Column chromatography was performed on Merck silica gel 60 (0.063-0.200 mm in diameter).

Fourier transform-infrared spectra (FT-IR) were recorded on Nicolet Impact 410 FT-IR spectrometer. The molecular weights of polymers were measured by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS), using α-cyano-4-hydroxycinnamic acid as the matrix. Neurotensin (MW = 1,674 Da) was used as a standard. MALDI-TOF-MS spectra were recorded on BULEX MALDI-TOF mass spectrometer. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 spectrometer operating at 400 MHz in $CDCl_3$ or DMSO-d_6 . Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane using the residue protonated solvent signal as a reference. Coupling constants (*J*) are for proton-proton coupling unless otherwise noted and are reported in hertz (Hz). UV spectrum was performed on a CARY 50 Prob UV-Visible Spectrophotometer (Varian Ltd.)

The elemental analysis was measured by CHN/O analyzer (Perkin Elmer PE2400 Series II). Thermogravimetric analysis (TGA) was measured by NETZSCH STA 409 C under nitrogen atmosphere. The heating rate is 20°C/min. Differential scanning calorimetry (DSC) was measured by Mettler Toledo DSC822^{e} under nitrogen atmosphere. The heating rate is 20°C/min.

2.4 Preparation of N^3-benzoylthymine



thymine N^3-benzoylthymine

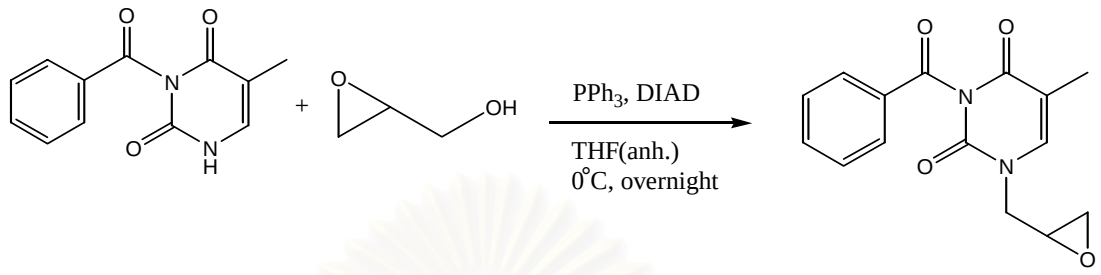
To a 100 mL round bottom flask was added thymine (1 equivalent) and pyridine, followed by addition of benzoylchloride (2.5 equivalents). The mixture was refluxed at 75 °C. The reaction was stopped at which time TLC indicated complete reaction. The mixture pH was then adjusted to 3 with 5% aqueous HCl and was extracted with ethyl acetate three times. The combined organic extracts were evaporated. K_2CO_3 (2.5 M in H_2O:dioxane 1:1) was added to the residue until the pH equaled 8. Then, the mixture was boiled at 70 °C. TLC was used to monitor the extent of reaction. Light-yellow solid was obtained by filtration. The solid was washed with cool water and the solvent was removed *in vacuo* to obtain the desired product.

Light yellow solid, 88% yield (1 mmol scale); ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.84 (3H, s, C$\underline{H}_3$), 7.55 (1H, s, C$\underline{H}$), 7.60 (2H, t, J =8.0 Hz, CH Ar), 7.80 (1H, t, J =7.6 Hz, C$\underline{H}$ Ar), 7.95 (2H, d, J =7.2 Hz, C$\underline{H}$ Ar); ^{13}C-NMR; δ 12.18 ($\underline{C}H_3$), 108.37 ($\underline{C}$H), 131.80 (olefinic-$\underline{C}$), 129.96, 130.69, 135.85, 139.27 ($\underline{C}_6H_5$ Ar), 150.42, 164.03, 170.62 (-$\underline{C}$=O).

2.5 Preparation of N^1-glycidyl-N^3-benzoylthymine

Two methods were used to prepare N^1-glycidyl-N^3-benzoylthymine. One is by the reaction of N^3-benzoylthymine with glycidol via Mitsunobu reaction. The other is by epoxidation of N^1-allyl-N^3-benzolythymine.

2.5.1 Mitsunobu reaction of N^3-benzoylthymine and glycidol



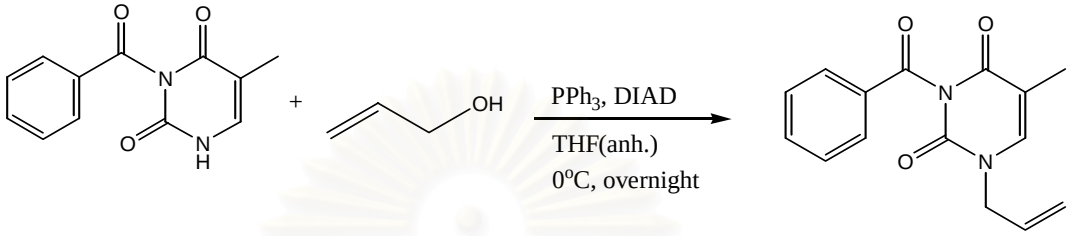
N^3-benzoylthymine glycidol N^1-glycidyl-N^3-benzoylthymine

To a 25 mL round bottom flask were added N^3-benzoylthymine (1 mmol) and triphenylphosphine (1.1 mmol), followed by addition of the anhydrous THF 6 mL under nitrogen atmostphere. Then, glycidol (1.1 mmol) was added, followed by addition of the DIAD (1.1 mmol) dropwise at 0°C. After leaving overnight, solvent was removed by evaporation. The residue was purified by column chromatography on silica gel using CH_2Cl_2-MeOH as an eluent.

White solid 0.2050 g, 70% yield (1 mmol scale); ^{1}H-NMR (400MHz, $CDCl_3$) δ 1.90 (3H, s, C$\underline{H}_3$), 2.55 (1H, dd, *J* =2.8, 4.4 Hz, C$\underline{H}_a$H$_b$-O), 2.83 (1H, t, J =4.4 Hz, CH$_a\underline{H}_b$-O), 3.22 (1H, m, C$\underline{H}$), 3.35 (1H, dd, *J* =6.8, 14.8 Hz, C$\underline{H}_a$H$_b$-N), 4.35 (1H, dd, *J* =2, 14.8 Hz, CH$_a\underline{H}_b$-N), 7.12 (1H, s, C$\underline{H}$-thymine), 7.42 (2H, t, *J* =8 Hz, C$\underline{H}$ Ar), 7.57 (1H, t, *J* =7.6 Hz, C$\underline{H}$ Ar), 7.85 (2H, d, *J* =8 Hz, C$\underline{H}$ Ar); ^{13}C-NMR; δ 12.45 (C$\underline{H}_3$), 45.11 (C$\underline{H}_2$-O), 49.69 (C$\underline{H}_2$-N), 49.91 (C$\underline{H}$-O), 110.96 (C$\underline{H}$=C), 129.23, 130.47, 135.17, 140.45 (C$_6\underline{H}_5$ Ar), 131.48 (olefinic–C), 149.91, 163.13, 168.93 (-C=O); IR (KBr); 761, 905, 1,248 (C-O st. of epoxide), 1,353, 1,446 (C=C st.). 1,645, 1,688, 1,738 (C=O).

2.5.2 Epoxidation of N^1-allyl-N^3-benzoylthymine

2.5.2.1 Preparation of N^1-allyl-N^3-benzolythymine

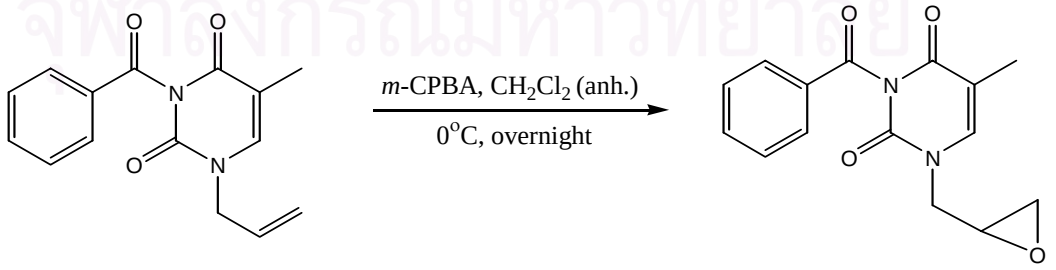


N^3-benzoylthymine allyl alcohol N^1-allyl-N^3-benzolythymine

To a 25 mL round bottom flask were added the N^3-benzoylthymine (1 mmol) and triphenylphosphine (1.1 mmol), followed by addition of the anhydrous THF 6 mL under nitrogen atmostphere. Then, allyl alcohol (1.1 mmol) was added, followed by addition of DIAD (1.1 mmol) dropwise at 0°C. After leaving overnight, solvent was removed by evaporation. The residue was purified by column chromatography on silica gel using hexane-EtOAc in 1:1 ratio as an eluent.

White solid 0.2322 g, 94.3% yield (1 mmol scale); ^{1}H-NMR (400MHz, $CDCl_3$) δ 1.97 (3H, s, C$\underline{H}_3$), 4.35 (2H, d, J =6.0 Hz, N-C$\underline{H}_2$), 5.31 (2H, HC=C$\underline{H}_2$), 5.90 (1H, m, C$\underline{H}$=CH$_2$), 7.08 (1H, s, C$\underline{H}$-thymine), 7.47 (2H, t, J =7.2, 15.6 Hz, C$\underline{H}_2$ Ar), 7.62 (1H, t, J =7.6, 15.2 Hz, C$\underline{H}$ Ar), 7.91 (2H, d, J =9.6 Hz, C$\underline{H}_2$ Ar).

2.5.2.2 Epoxidation of N^1-allyl-N^3-benzoylthymine

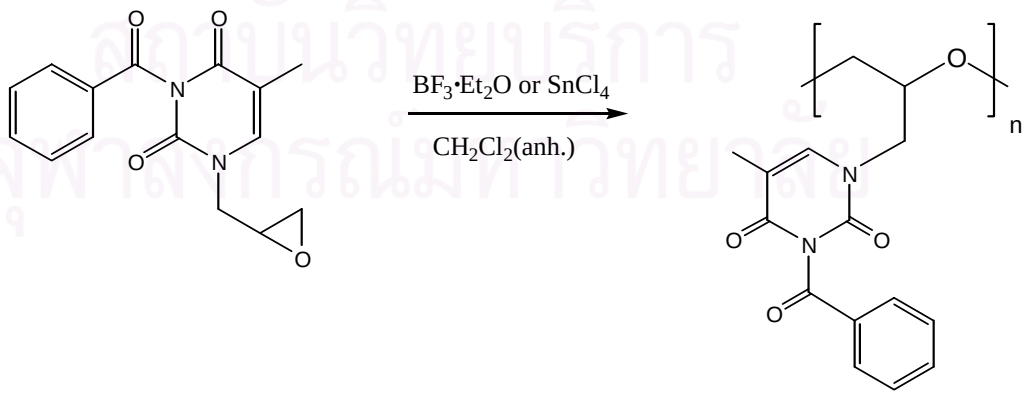


N^1-allyl-N^3-benzolythymine N^1-glycidyl-N^3-benzoylthymine

To a 25 mL round bottom flask were added the N^1-allyl-N^3-benzolythymine (0.1 mmol) and *m*-CPBA (0.3 mmol), followed by addition of anhydrous CH_2Cl_2 under nitrogen atmosphere. The mixture was stirred for 24 hours at 0°C. After the reaction was completed, the mixture was extracted with diethyl ether three times. The combined extracts were washed with saturated solution of $NaHCO_3$ and brine, respectively. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed by evaporation. The residue was purified by column chromatography on silica gel using hexane-ethyl acetate in 1:2 ratios as solvent.

White solid 0.0050 g, 17 % yield (0.1 mmol scale); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.90 (3H, s, C<u>H</u>$_3$), 2.55 (1H, dd, *J* =2.8, 4.4 Hz, C<u>H</u>$_a$H$_b$-O), 2.83 (1H, t, J =4.4 Hz, CH$_a$<u>H</u>$_b$-O), 3.22 (1H, m, C<u>H</u>), 3.35 (1H, dd, *J* =6.8, 14.8 Hz, C<u>H</u>$_a$H$_b$-N), 4.35 (1H, dd, *J* =2, 14.8 Hz, CH$_a$<u>H</u>$_b$-N), 7.12 (1H, s, C<u>H</u>-thymine), 7.42 (2H, t, *J* =8 Hz, C<u>H</u> Ar), 7.57 (1H, t, *J* =7.6 Hz, C<u>H</u> Ar), 7.85 (2H, d, *J* =8 Hz, C<u>H</u> Ar);^{13}C-NMR; δ 12.45 (<u>C</u>H$_3$), 45.11 (<u>C</u>H$_2$-O), 49.69 (<u>C</u>H$_2$-N), 49.91 (<u>C</u>H-O), 110.96 (<u>C</u>H=C), 129.23, 130.47, 135.17, 140.45 (<u>C</u>$_6$H$_5$ Ar), 131.48 (olefinic–<u>C</u>), 149.91, 163.13, 168.93 (-<u>C</u>=O).

2.6 Polymerization of N^1-glycidyl-N^3-benzoylthymine



N^1-glycidyl-N^3-benzoylthymine poly(N^1-glycidyl-N^3-benzoylthymine)

The reactions were set under nitrogen atmosphere using a glove bag. To a 25 mL round bottom flask, N^1-glycidyl-N^3-benzoylthymine (1 equivalent) was dissolved in anhydrous CH_2Cl_2. The catalyst (8% mol of monomer) was added to the monomer solution. The mixture was stirred at room temperature under nitrogen atmosphere. After 7 days, the reaction was terminated. The CH_2Cl_2 was removed by evaporation. Then, crude product was dissolved in DMSO and precipitated in distilled water. See Table 2.1 for details.

^{1}H-NMR (400MHz, DMSO-d_6) δ 1.83 (3H, m, C$\underline{H}_3$-thymine), 3.35, 3.49, 3.97 (3H, m, C$\underline{H}_2$-C$\underline{H}$-O), 4.23, 4.50 (2H, br, C$\underline{H}_2$-N), 4.71, 5.44, 5.80 (3H, br, C$\underline{H}$-OH, C$\underline{H}_2$-OH), 7.38-7.94 (6H, m, C$\underline{H}$-thymine and $C_6\underline{H}_5$-Ar); ^{13}C-NMR; δ 13.07 ($\underline{C}H_3$-thymine), 44.42-55.24 ($\underline{C}H_2$-N), 60.75-69.42 (-O-$\underline{C}$H-$\underline{C}H_2$), 108.18 ($\underline{C}$H=C), 129.10-135.99 ($\underline{C}_6H_5$-Ar), 133.91, 143.43-144.20 (olefinic-$\underline{C}$), 149.88-170.26 ($\underline{C}$=O); IR (KBr); 1,081 (C-O st.), 1,260 (C-N st.), 1,376, 1,443 (C=C st.), 1,563 (N-H bend), 1,645, 1,692, 1,738 (C=O st.), 3,443 (-OH st.).

Table 2.1 The results of polymerization.

Entry	Initiator		Temp.	Time	Polymerization	appearance
	type	%mol	(°C)	(days)	yield (%)	
1	$BF_3{\cdot}Et_2O$	1	rt	7	trace	White solid
2	$BF_3{\cdot}Et_2O$	4	rt	7	39	White solid
3	$BF_3{\cdot}Et_2O$	8	-16	2	0	-
4	$BF_3{\cdot}Et_2O$	8	rt	2	59	White solid
5	$BF_3{\cdot}Et_2O$	8	rt	5	63	White solid
6	$BF_3{\cdot}Et_2O$	8	rt	7	80	White solid
7	$BF_3{\cdot}Et_2O$	24	rt	7	54	White solid
8	$SnCl_4$	8	rt	7	50	White solid

2.7 Deprotection of poly (N^1-glycidyl-N^3-benzoylthymine)

The benzoyl protecting group was removed by stirring poly(N^1-glycidyl-N^3-benzoylthymine) with aq. NH_3:MeOH (1:1) at 90°C for overnight. Distilled water was added to the mixture following by extraction with CH_2Cl_2 three times. The remaining aqueous layer was evaporated to obtain a product that was further dried in *vacuo* to obtain the desired polymer. See Table 2.2 for details.

White solid 0.2184 g, 95.5 % yield (1 mmol scale); ^{1}H-NMR (400 MHz, D_2O) δ 1.76 (3H, m, C<u>H</u>$_3$-thymine), 3.43-4.04 (5H, br, -C<u>H</u>$_2$C<u>H</u>(C<u>H</u>$_2$-N-)O- and C<u>H</u> (C<u>H</u>$_2$-N-)C<u>H</u>$_2$O-), 7.24-7.71 (6H, m, C<u>H</u>-thymine and C_6H_5-Ar); ^{13}C-NMR; δ 11.20-11.94 (CH_3-thymine), 44.22-51.91 (CH_2-N), 62.78-72.12 (-O-CH-CH_2-), 103.29-110.18 (CH=C-), 127.19-145.63 (C_6H_5-Ar), 143.60 (olefinic-<u>C</u>), 152.16-174.70 (C=O); IR (KBr); 1,088 (C-O st.), 1,396 (C-N st.), 1,563 (N-H bend), 1,668 (C=O st.), 3,450 (C-O st.)

Table 2.2 The results of deprotection.

Entry	Initiator		Temp. (°C)	Time (days)	Deprotection yield (%)	Overall yield	appearance
	type	%mol					
1	$BF_3{\cdot}Et_2O$	1	rt	7	N/A	N/A	White solid
2	$BF_3{\cdot}Et_2O$	4	rt	7	83	32	White solid
3	$BF_3{\cdot}Et_2O$	8	-16	2	N/A	N/A	-
4	$BF_3{\cdot}Et_2O$	8	rt	2	94	55	White solid
5	$BF_3{\cdot}Et_2O$	8	rt	5	95	60	White solid
6	$BF_3{\cdot}Et_2O$	8	rt	7	95	76	White solid
7	$BF_3{\cdot}Et_2O$	24	rt	7	98	53	White solid
8	$SnCl_4$	8	rt	7	89	45	White solid

N/A not available

2.8 Molecular weight determination

Weight-average molecular weight ($\overline{M}_w$), number-average molecular weight ($\overline{M}_n$) and polydispersity index (PDI) are defined as follows.

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i}$$

$$\overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

where as m_i = mass

n_i = signal intensity of i^{th} oligomers obtained from MALDI-TOF MS

Data examples from MS analysis of thymine oligomer (Figure 3.14 (a)).

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
388.02	1.51×10^5	1.02×10^4	3.95×10^6	1.53×10^9
569.43	3.24×10^5	8.56×10^3	4.87×10^6	2.77×10^9
750.62	5.63×10^5	2.84×10^3	2.13×10^6	1.60×10^9
931.88	8.68×10^5	1.24×10^3	1.16×10^6	1.08×10^9
1112.60	1.24×10^6	3.82×10^2	4.25×10^5	4.73×10^8
1293.06	1.67×10^6	1.68×10^2	2.17×10^5	2.81×10^8
1473.16	2.17×10^6	8.27×10^1	1.22×10^5	1.79×10^8
1652.91	2.73×10^6	5.12×10^1	8.47×10^4	1.40×10^8
		$\sum n_i = 2.35 \times10^4$	$\sum m_i n_i = 1.30\times 10^7$	$\sum m_i^2 n_i = 8.06 \times 10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i}$$

$$\overline{M}_w = \frac{8.06 \times 10^9}{1.30 \times 10^7}$$

$$\overline{M}_w = 6.22 \times 10^2$$

$$\overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_n = \frac{1.30 \times 10^7}{2.35 \times 10^4}$$

$$\overline{M}_n = 5.51 \times 10^2$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

$$PDI = \frac{6.22 \times 10^2}{5.51 \times 10^2}$$

$$PDI = 1.13$$

2.9 Biophysical Studies

The nucleic acids were used as received without further treatment. The concentration of oligonucleotide, nucleic acids and thymine oligomer solutions was determined from the absorbance at 260 nm. The molar extinction coefficients at 260 nm (ε_{260}) of 8.8 mL.μmol^{-1}.cm^{-1} and 10.8 mL.μmol^{-1}.cm^{-1} was used for thymine and adenine respectively.

Interaction of poly(N^1-glycidylthymine) with nucleic acid

Melting temperature (T_m) experiments were performed in order to determine interaction between poly(N^1-glycidylthymine) and polynucleotide. A CARY 100 UV Spectrophotometer (Varian Ltd.) equipped with a thermal melt system was employed. The sample was prepared by mixing calculated amounts of stock oligonucleotide and poly(N^1-glycidylthymine) solution together in sodium phosphate buffer (pH 7.0). The final volumes were adjusted to 3.0 mL by adding deionized water. The samples were transferred to a 10 mm quartz cell with teflon stopper and equilibrated at the starting temperature for at least 30 min. The OD_{260} was recorded in steps from 20-90 °C (block temperature) with a temperature increment of 1 °C/min. The results were normalized by dividing the absorbance at each temperature by the initial absorbance. Correct temperature obtained by comparison of the actual and set temperature and normalized absorbance are defined as follows.

$$\text{Correct. Temp.} = (0.978 \times T_{obs}) - 0.6068$$

$$\text{Normalized Abs.} = Abs_{obs}/Abs_{init}$$

Data examples from UV analysis of thymine oligomer & Poly(dA) (Figure 3.18).

Entry	Thymine oligomer&Poly(dA) 20.00-90.00°C (Ramp 3)			
	Temperature (°C)	Absorbance	Correct Temp (°C)	Normalized Abs
1	20.4200	0.1808	19.3640	1.000
2	21.4200	0.1809	20.3420	1.000
3	22.3700	0.1809	21.2711	1.000
4	23.3700	0.1809	22.2491	1.000
5	24.3700	0.1810	23.2271	1.001
6	25.3700	0.1809	24.2051	1.001
7	26.3700	0.1811	25.1831	1.001
8	27.3700	0.1811	26.1611	1.002
9	28.4200	0.1811	27.1880	1.002
10	29.4200	0.1812	28.1660	1.002

For example

In entry 1; T_{obs} = 20.4200 °C, Abs_{init} = 0.1808, Abs_{obs} = 0.1809;

Correct. Temp. = $(0.978 \times T_{obs}) - 0.6068$

Correct. Temp. = $(0.978 \times 20.4200) - 0.6068$

= 19.3640°C

Normalized Abs. = Abs_{obs}/Abs_{init}

= 0.1809/0.1808

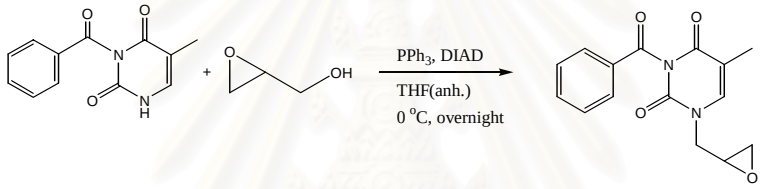
= 1.000

CHAPTER III

RESULTS AND DISCUSSION

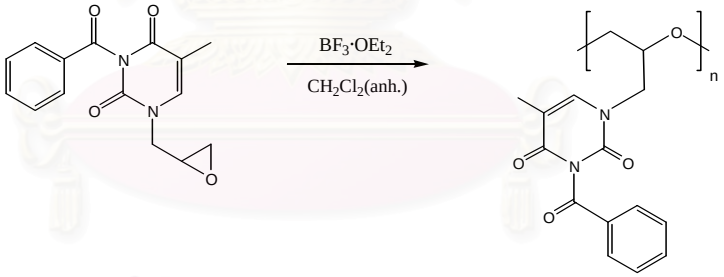
This chapter is divided into 2 parts. The first part shows results of the synthesis of thymine-containing polymer (scheme 3.1). In the second part, thermal and biophysical properties of the polymers are given.

Monomer synthesis



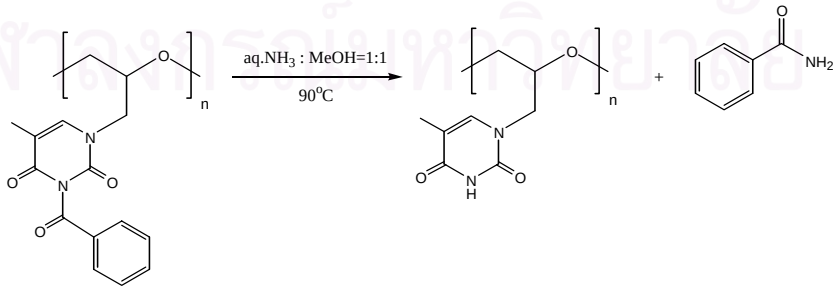
N^3-benzoylthymine glycidol N^1-glycidyl-N^3-benzoylthymine

Polymerization step



N^1-glycidyl-N^3-benzoylthymine poly(N^1-glycidyl-N^3-benzoylthymine)

Deprotection step

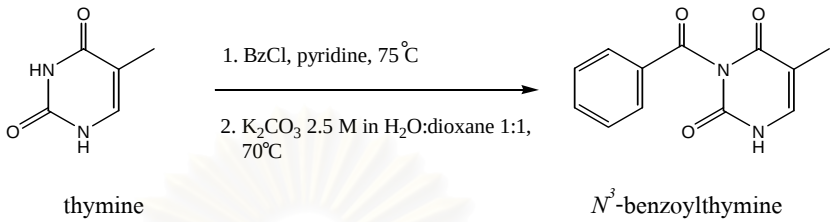


poly(N^1-glycidyl-N^3-benzoylthymine) poly(N^1-glycidylthymine) benzamide

Scheme 3.1 Three steps synthesis of thymine-containing polyether

3.1 Synthesis of thymine containing polymer

3.1.1 Synthesis of monomer



Scheme 3.2 Synthesis of N^3-benzoylthymine

The cyclic ether monomer consisting of thymine was started from N^3-benzoylthymine (Scheme 3.2). The reaction gave light yellow solid in 88% yield. The structure of N^3-benzoylthymine was verified by NMR (Figure 3.1). The ^{1}H-NMR shows signals of CH_3 and CH of thymine at 1.84 and 7.55 ppm respectively. The signals of five aromatic protons are at 7.60, 7.80 and 7.95 ppm. The ^{13}C-NMR is shown in Figure 3.2. The signal of $\underline{C}H_3$, C=$\underline{C}$H and $\underline{C}$=CH of thymine are at 12.30, 108.14 and 138.20 ppm respectively. The signals of two carbonyl groups of thymine are at 150.42 and 164.03 ppm. The carbonyl of benzoyl group is at 170.62 ppm.

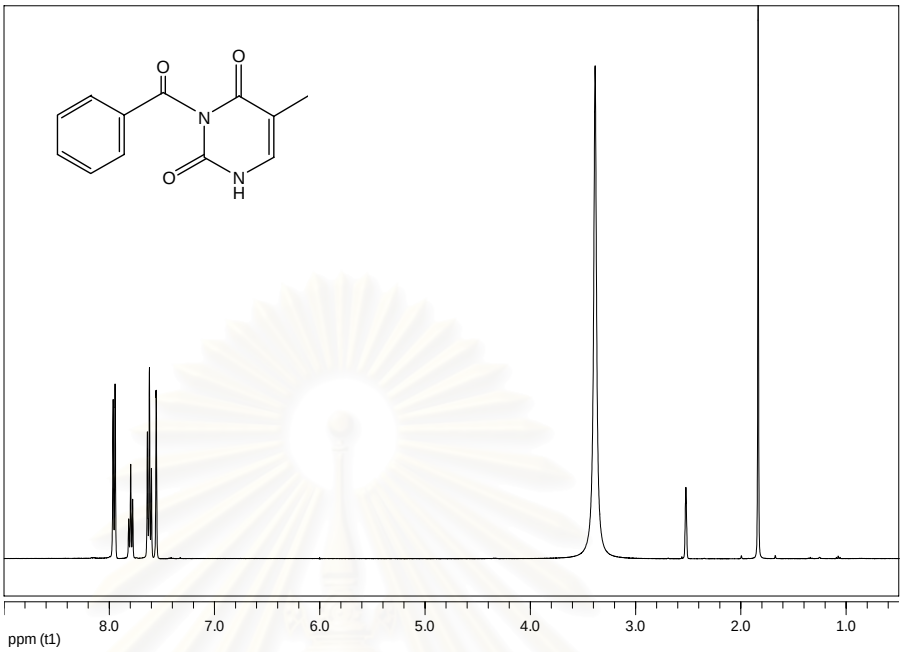


Figure 3.1 ^{1}H NMR of N^{3}-benzoyl thymine (DMSO-d_6, 400 MHz, 25°C)

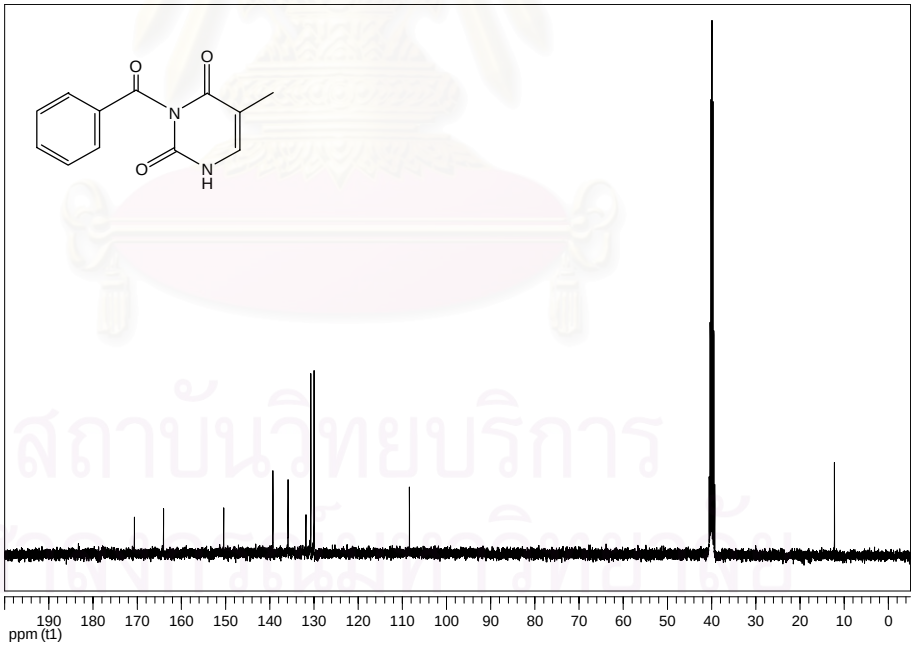
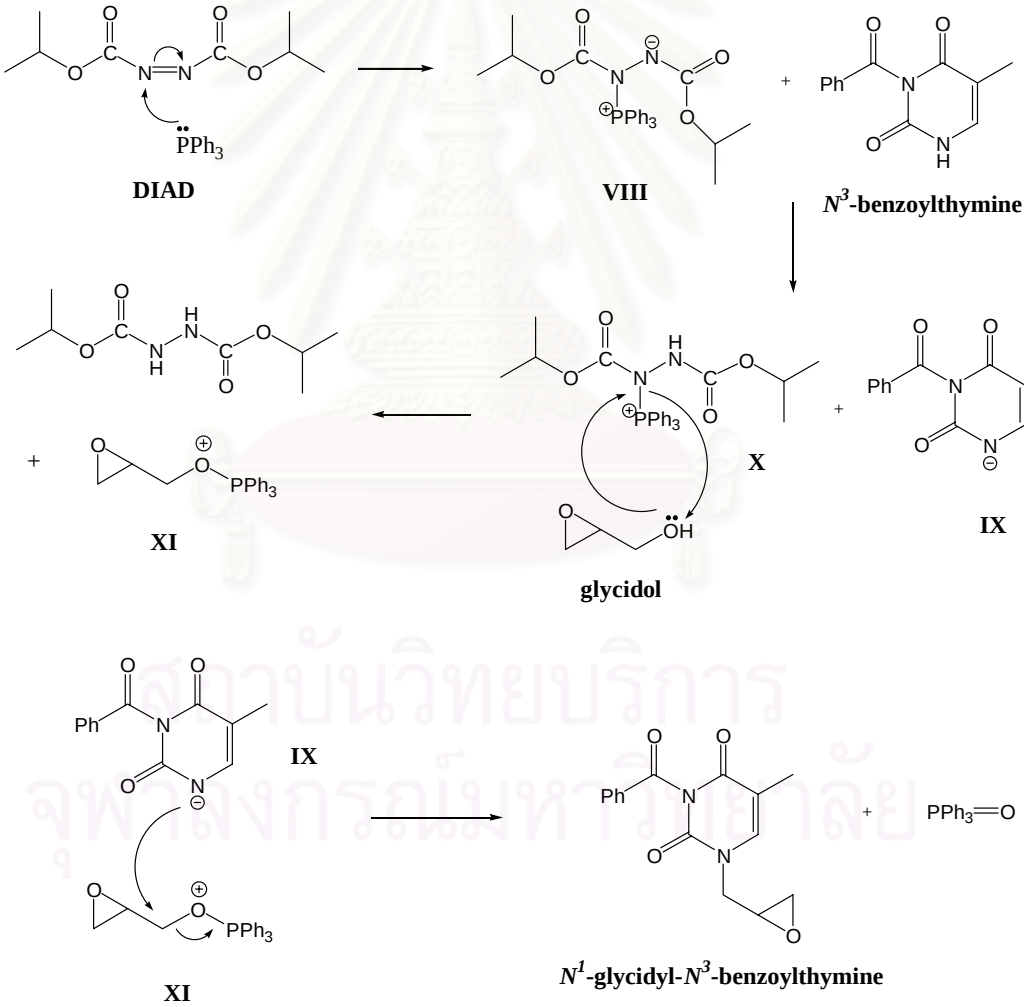


Figure 3.2 ^{13}C NMR of N^{3}-benzoyl thymine (DMSO-d_6, 400 MHz, 25°C)

The N^{1}-glycidyl-N^{3}-benzoylthymine monomer was synthesized by two methods. In the first method N^{3}-benzoylthymine was reacted with glycidol by Mitsunobu reaction. This reaction was carried out at 0°C in anhydrous THF. The azo

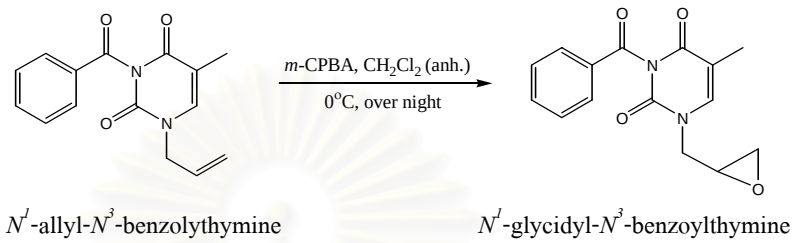
compound, diisopropyl azodicarboxylate (DIAD) was used as an electrophile, coupled with PPh_3. The reaction gave white solid product in 70% yield.

The mechanism (Scheme 3.3) involves attacks of the phosphorous atom on PPh_3 on the N-atom in DIAD, resulting in reactive species **VIII** capable of abstracting a proton at N-H position in N^3-benzoylthymine. Reaction products are the nucleophile (**IX**) and the positive species (**X**), which consequently interact with each other to form **XI**. The compound **XI** further reacts with **IX** to form N^1-glycidyl-N^3-benzoylthymine and $Ph_3P{=}O$.



Scheme 3.3 The mechanism of Mitsunobu reaction.

In the second method, N^1-glycidyl-N^3-benzolythymine was synthesized from epoxidation of N^1-allyl-N^3-benzolythymine, using *m*-CPBA as an epoxidizing agent (Scheme 3.4). This reaction gave the desired product in only 17 % yield.



N^1-allyl-N^3-benzolythymine N^1-glycidyl-N^3-benzoylthymine

Scheme 3.4 Epoxidation of N^1-allyl-N^3-benzolythymine

Comparing between two methods, the Mitsunobu reaction gave higher yield than the epoxidation pathway. Therefore, the first method was chosen for the synthesis of N^1-glycidyl-N^3-benzoylthymine monomer.

The structure of N^1-glycidyl-N^3-benzoylthymine was verified by NMR (Figures 3.3-3.4), IR and elemental analysis. The IR spectrum of N^1-glycidyl-N^3-benzoylthymine is shown in Figure 3.5. The signals of two C=O of thymine are at wave number 1,738 and 1,688 cm^{-1}. The signal at 1,645 cm^{-1} belongs to C=O at benzoyl group. The signal of aromatic C=C stretching is at 1,356 and 1,446 cm^{-1}. The epoxide signal (C-O stretching) are at 1248, 905 and 761 cm^{-1}.

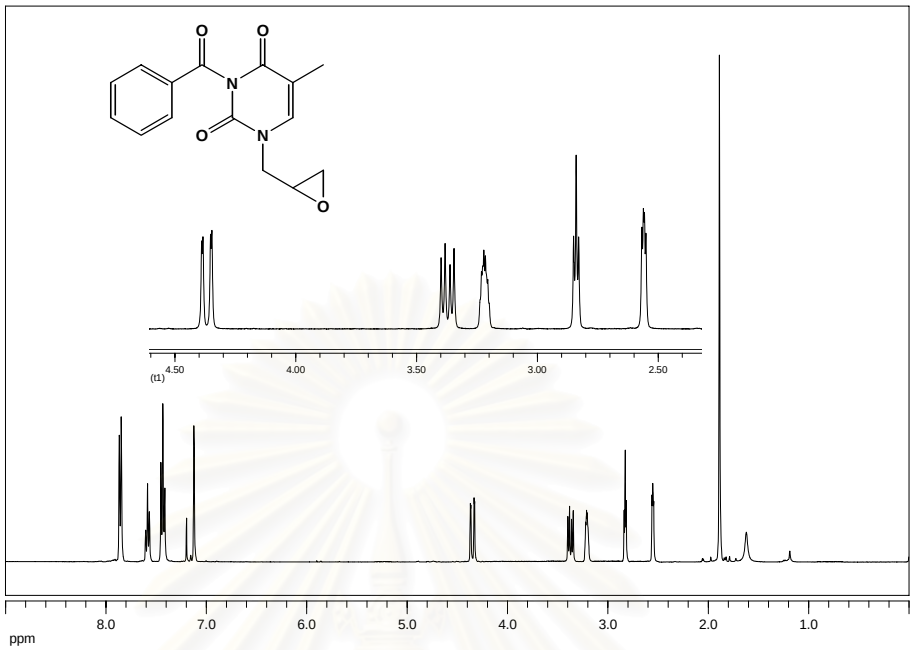


Figure 3.3 ^{1}H NMR of *N*^{1}-glycidyl-*N*^{3}-benzoylthymine ($CDCl_3$, 400 MHz, 25°C)

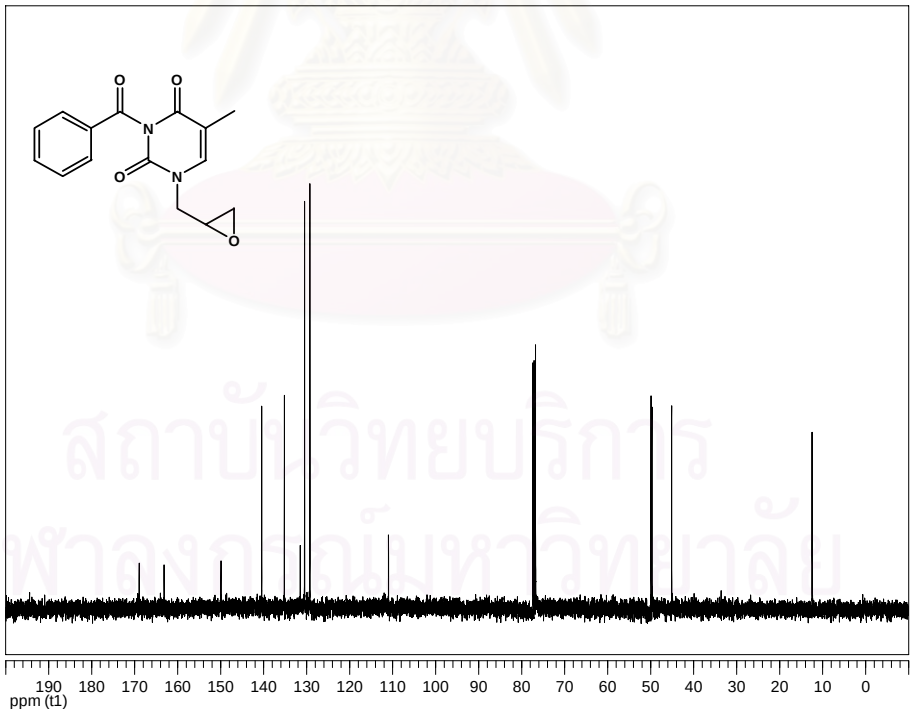


Figure 3.4 ^{13}C NMR of *N*^{1}-glycidyl-*N*^{3}-benzoylthymine ($CDCl_3$, 400 MHz, 25°C)

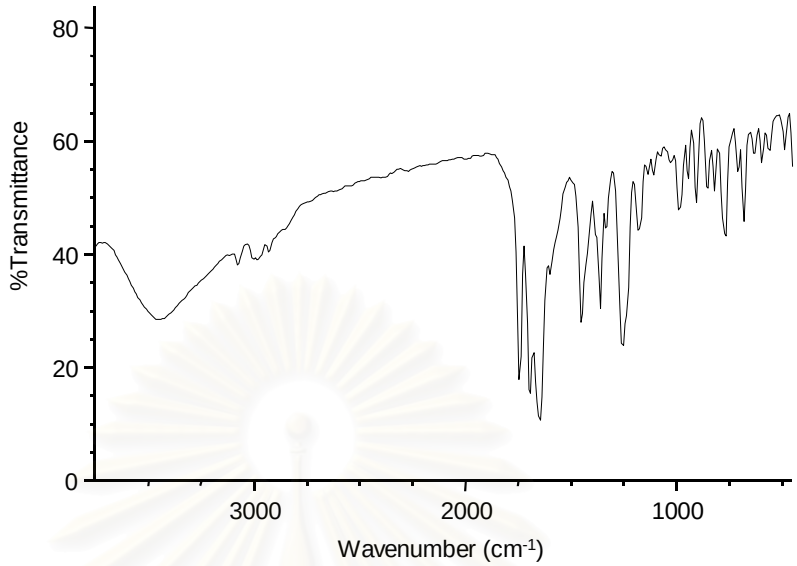


Figure 3.5 IR spectrum of N^1-glycidyl-N^3-benzoylthymine (KBr pellet)

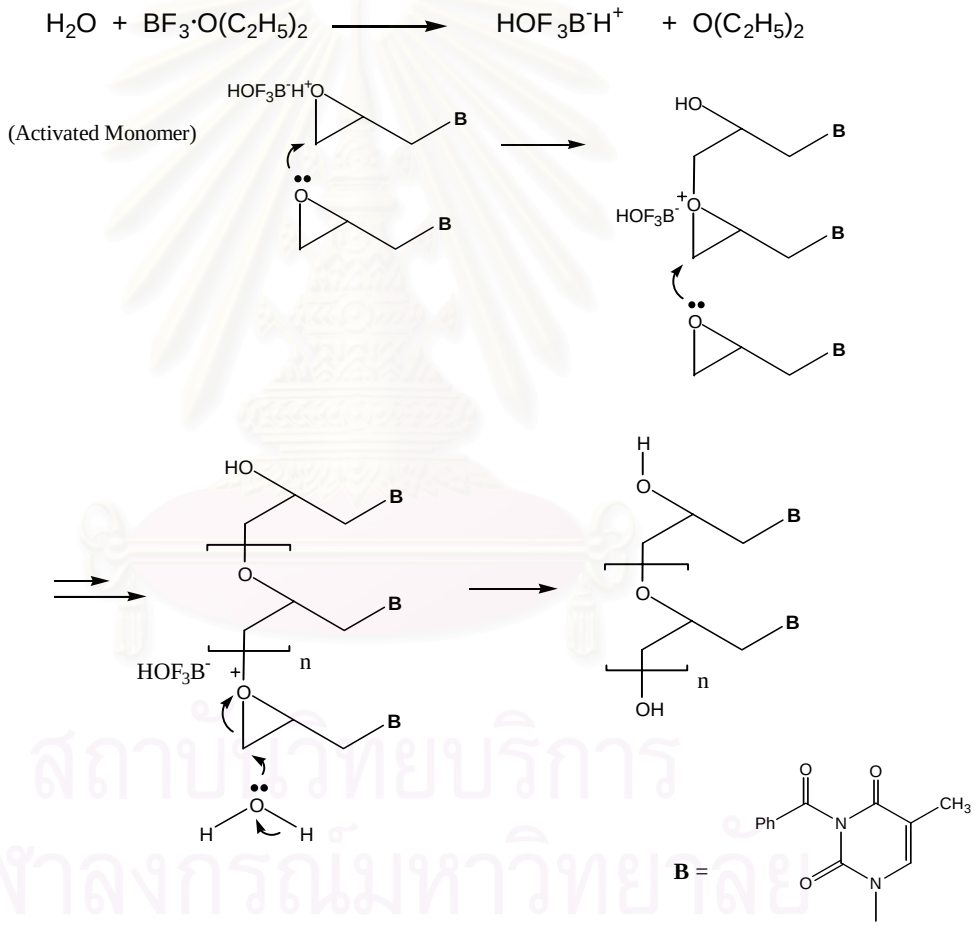
The elemental analysis of N^1-glycidyl-N^3-benzoylthymine is shown in Table 3.1. The obtained atomic composition of N^1-glycidyl-N^3-benzoylthymine was almost equal to the analytical calculated (Anal. Calcd.) value.

Table 3.1 Elemental analysis of N^1-glycidyl-N^3-benzoylthymine ($C_{15}H_{14}N_2O_3$)

% Element	Anal. Calcd.	Found
C	62.93	63.22
H	4.93	4.66
N	9.78	9.78

3.1.2 Polymerization of N^1-glycidyl-N^3-benzoylthymine

The epoxide group of N^1-glycidyl-N^3-benzoylthymine is susceptible to ring-open by a nucleophile presence in the system. In this study $BF_3{\cdot}Et_2O$ is used as an initiator similar to that reported by Dworak *et al*. [15], and Sohn *et al*. [17]. After polymerization, the benzoyl protecting group on the resulting polymer was then removed under basic condition (aq. NH_3:MeOH=1:1 v/v).



Scheme 3.5 Mechanism of cationic ring-opening polymerization

The polymerization mechanism involves cationic ring-opening of the epoxide on the N^1-glycidyl-N^3-benzoylthymine (Scheme 3.5). A protic compound such as water is often required as co-initiator, interacting with Lewis acids like $BF_3 \cdot Et_2O$. In the next step, protonation of the monomer produces secondary oxonium ion (activated monomer) [16]. After that, the nucleophilic oxygen of the other molecule attacks the neighboring carbon in the activated monomer. The epoxide ring then opens and gives hydroxyl terminated macro-monomers which in turn undergo further reaction with other monomer. These steps repeat until the reaction is terminated or the monomer has run out. The termination was occurred by nucleophiles such as H_2O at the end of the polymer chain. Hydroxyl terminated polymers are thus obtained.

The variation of polymerization condition is shown in Table 3.2. The obtained product in every condition gave the same results. Then, the results of entry 4 are shown in all Figures.

Table 3.2 The variation of polymerization condition for synthesis of Poly(N^1-glycidyl-N^3-benzoylthymine).

Entry	Initiator		Temp. (°C)	Time (days)
	type	%mol		
1	$BF_3 \cdot Et_2O$	1	rt	7
2	$BF_3 \cdot Et_2O$	4	rt	7
3	$BF_3 \cdot Et_2O$	8	-16	2
4	$BF_3 \cdot Et_2O$	8	rt	2
5	$BF_3 \cdot Et_2O$	8	rt	5
6	$BF_3 \cdot Et_2O$	8	rt	7
7	$BF_3 \cdot Et_2O$	24	rt	7
8	$SnCl_4$	8	rt	7

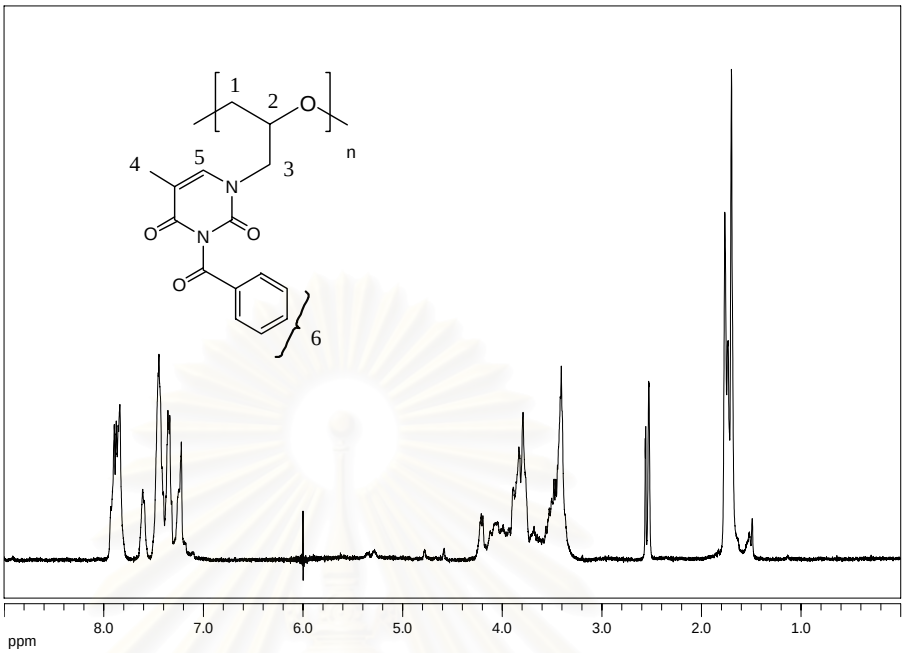


Figure 3.6 ^{1}H NMR of poly(N^{1}-glycidyl-N^{3}-benzoylthymine) (DMSO-d_6, 400 MHz, 25°C)

The structure of polymerization product [poly(N^{1}-glycidyl-N^{3}-benzoylthymine)] was verified by NMR (Figure 3.6). Figure 3.6 shows ^{1}H-NMR of poly(N^{1}-glycidyl-N^{3}-benzoylthymine). The signals of ether protons are at 3.2-4.2 ppm. The C$\underline{H}_3$ and C$\underline{H}$ of thymine are at 1.7 and 7.2 ppm respectively. The signals of five aromatic protons are at 7.4-7.7 ppm. Analysis of NMR signals can be discussed as follows. From Table 3.2, the peak area ratio of combined five protons at 1, 2 and 3 positions (ether proton and CH_2-N) and the 3H of CH_3 of thymine are 5.2:3.0, close to the expected 5:3. The peak area ratio of signal of CH_3 and CH-thymine at position 4 and 5 are 3.0:0.7 that extrapolate close to the expected 3.0-1.0. However, the combined area of proton signals at position 6 (phenyl proton) is only 4.1, much lower than the expected 5H. This result suggests that some of the aromatic protons are absent. It's possible that some benzoyl protecting groups were removed in acidic condition during polymerization. In order to elucidate this observation, N^{3}-benzoyl thymine, a model monomer without the epoxide, was tested under the identical

polymerization condition. It was found that the integration ratio of aromatic protons and CH-thymine had decreased as well (Figure 3.7). It is therefore proposed here that the benzoyl protecting group is at least partly removed during the polymerization step.

Table 3.3 NMR Data for poly(N^1-glycidyl-N^3-benzoylthymine) in DMSO-d_6

Position	number of protons	δ_H (J in Hz)	Peak Integration
1, 2, 3	5H	3.2-4.2	5.2
4	3H	1.70	3.0
5	1H	7.2	0.7
6	5H	7.3-7.9	4.1

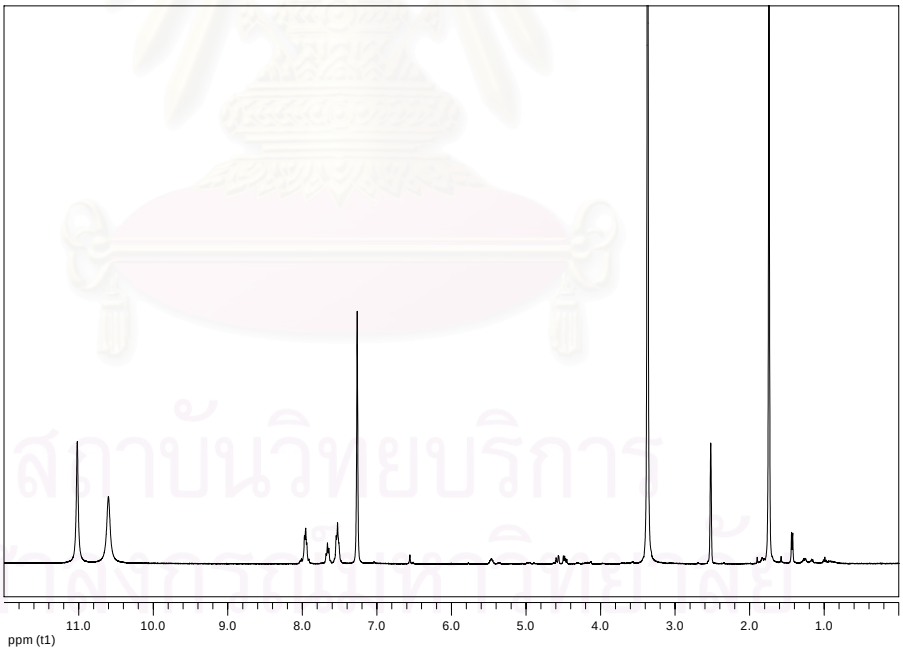


Figure 3.7 ^{1}H NMR of N^3-benzoylthymine in the presence of BF_3 (DMSO-d_6, 400 MHz, 25°C)

The ^{13}C-NMR spectrum of poly(N^1-glycidyl-N^3-benzoylthymine) is shown in Figure 3.8. The signals of $\underline{C}H_2$-N and $\underline{C}H_2$-$\underline{C}$H-O are at 44-55 and 60-70 ppm respectively. The signal of $\underline{C}H_3$, $\underline{C}$H=C and 3°-$\underline{C}$ are at 12, 110 and 133 ppm respectively. The signals at 150-170 ppm belonged to the three C=O groups. The five aromatic carbons are at 130-140 ppm.

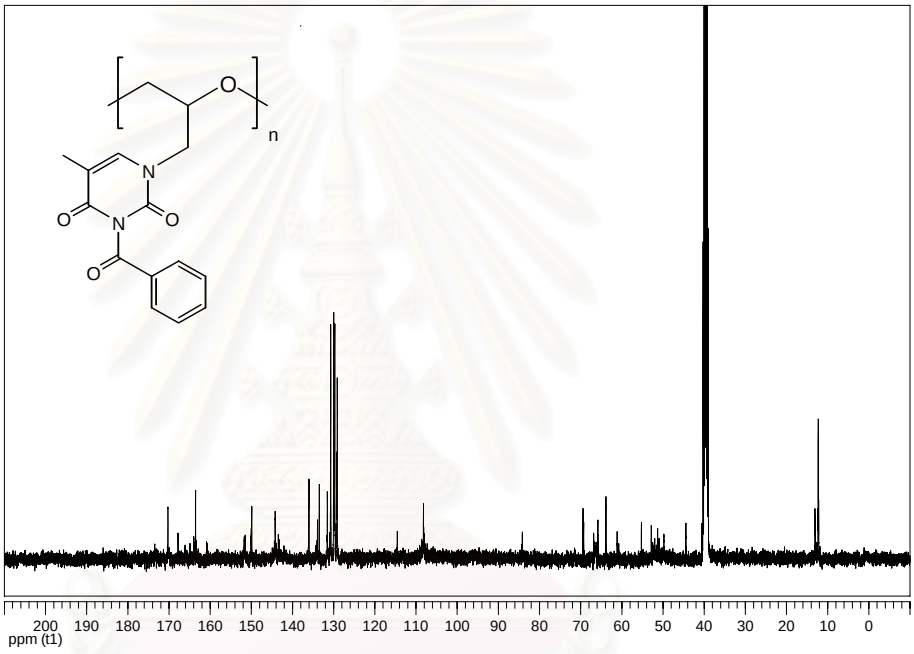


Figure 3.8 ^{13}C NMR of poly (N^1-glycidyl-N^3-benzoylthymine) (DMSO-d_6, 400 MHz, 25°C)

The IR spectrum of poly(N^1-glycidyl-N^3-benzoylthymine) is shown in Figure 3.9. The signals of epoxide (1,248, 905 and 761 cm^{-1}) disappear from the IR spectrum of the polymer. Instead, C-O stretching signal of ether appears at 1,081 cm^{-1}. The other signals remain the same. The signals of two carbonyl group of thymine are at wave number 1,738 and 1,692 cm^{-1}. The signal at 1,645 cm^{-1} belongs to C=O of benzoyl group. The signal of N-H bending is at 1,563 cm^{-1}. The signals of C=C stretching are at 1,443 and 1,376 cm^{-1}. The signal of C-N stretching is at 1,260 cm^{-1}.

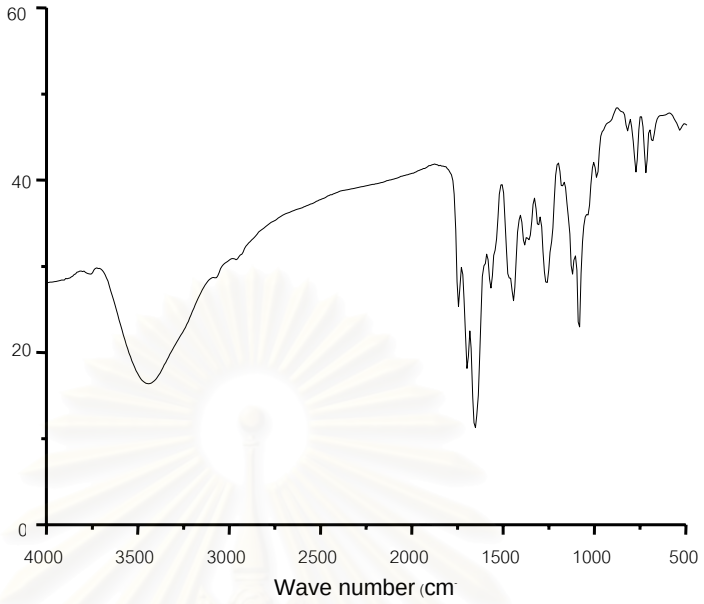


Figure 3.9 IR spectrum of poly(N^1-glycidyl-N^3-benzoylthymine) (KBr pellet)

3.1.3 Deprotection step for poly(N^1-glycidyl-N^3-benzoylthymine)

The benzoyl protecting group was removed from poly(N^1-glycidyl-N^3-benzoylthymine) in basic condition. The structure of poly(N^1-glycidylthymine) was determined by NMR and IR. The ^{1}H-NMR and ^{13}C-NMR are shown in Figures 3.10 and 3.11 respectively. The signals of ether proton were at 3.2-4.0 ppm, and the CH_3 and CH of thymine were at 1.7 and 7.2 ppm. However, the signals for five phenyl protons that belonged to the benzoyl protecting group remained present at 7.1-7.7 ppm, overlapping with CH-thymine. From Table 3.4, the peak area integrations of all protons are not different from poly(N^1-glycidyl-N^3-benzoylthymine). The signal for the benzoyl protecting group remained, even after was purified by stirring in ether. This is probably due to adsorption of the benzamide within the polymer matrix. The disconnection between the protecting group and the thymine unit has been confirmed by several techniques - see belows.

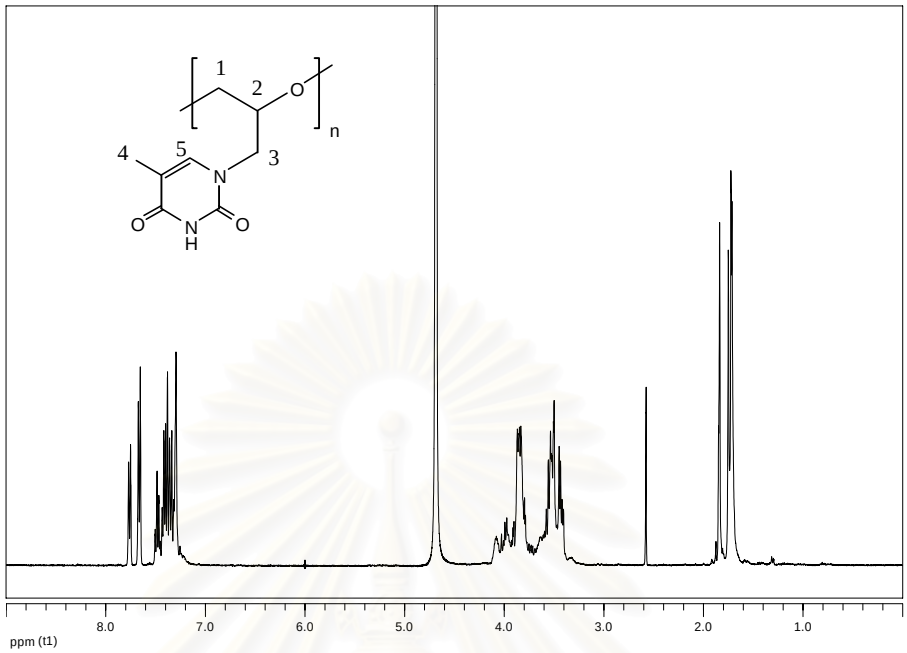


Figure 3.10 ^{1}H NMR of poly (N^{1}-glycidylthymine) (D_2O, 400 MHz, 25°C)

Table 3.4 ^{1}H NMR Data for poly(N^{1}-glycidylthymine) in DMSO-d_6

Position	Number of Protons	δ_H (J in Hz)	Peak Integration	Comment
1, 2, 3	5H	3.2-4.2	5.35	-
4	3H	1.70	3.00	-
5	1H	7.3	0.7	Position at 5 was overlapped with aromatic protons of by-product

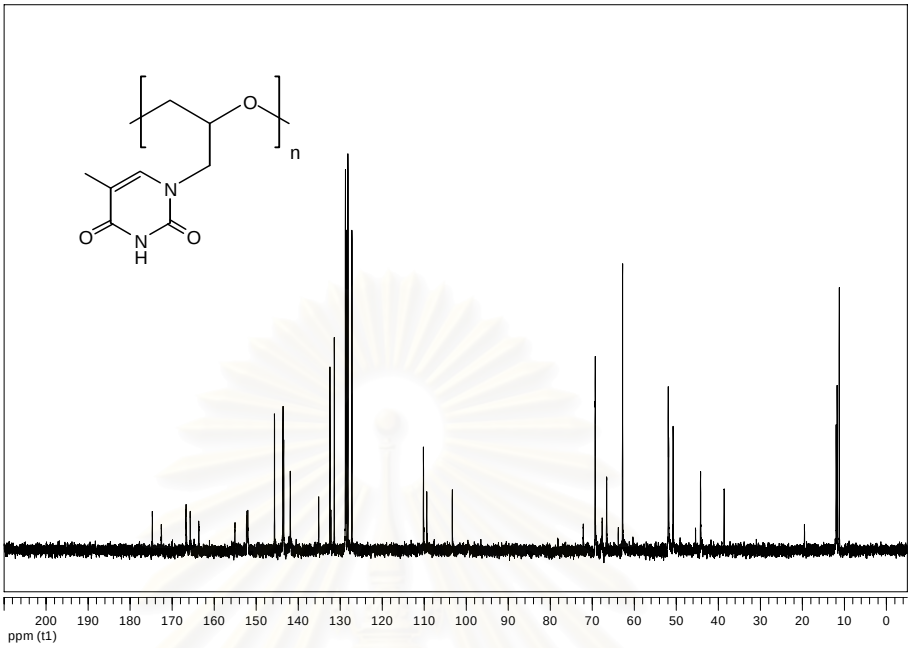


Figure 3.11 ^{13}C NMR of poly(N^1-glycidylthymine) (D_2O, 400 MHz, 25°C)

The IR spectrum of poly(N^1-glycidylthymine) is shown in Figure 3.12. The signal of C=O stretching is at wave number 1,668 cm^{-1}. The signal of N-H bending is at 1,563 cm^{-1}. The signal of C-N stretching is at 1,396 cm^{-1}. The C-O stretching of ether is present at 1,088 cm^{-1}. The signal of aromatic out of plane bending (800-700 cm^{-1}) disappears in IR spectrum of poly(N^1-glycidylthymine).

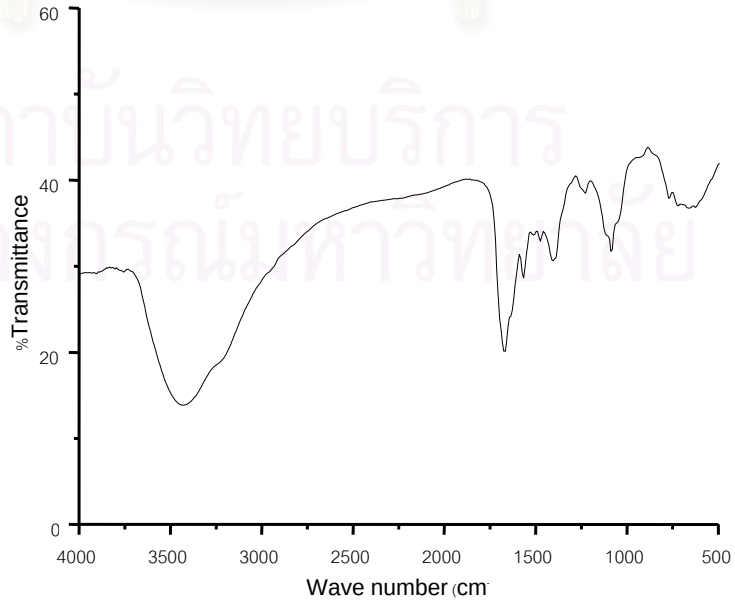


Figure 3.12 FT-IR spectrum of poly(N^1-glycidylthymine) (KBr pellet)

Results from polymerization and deprotection parts shown above confirm that the thymine containing polymer can be synthesized successfully by using BF_3 as an initiator. Structural information was obtained using FT-IR and NMR spectroscopy.

3.1.4 Optimization of polymerization condition

Table 3.5 Polymerization of N^1-glycidyl-N^3-benzoylthymine in the presence of $BF_3{\cdot}Et_2O$ and $SnCl_4$.

Entry	Initiator		Temp. (°C)	Time (days)	Overall yield
	type	%mol			
1	$BF_3{\cdot}Et_2O$	1	rt	7	N/A
2	$BF_3{\cdot}Et_2O$	4	rt	7	32
3	$BF_3{\cdot}Et_2O$	8	-16	2	0
4	$BF_3{\cdot}Et_2O$	8	rt	2	55
5	$BF_3{\cdot}Et_2O$	8	rt	5	60
6	$BF_3{\cdot}Et_2O$	8	rt	7	76
7	$BF_3{\cdot}Et_2O$	24	rt	7	53
8	$SnCl_4$	8	rt	7	45

N/A not available

Table 3.5 shows the details of polymerization. In Entry 1, 1% mol of $BF_3{\cdot}Et_2O$ was used in the polymerization set at room temperature for 2 days. But the polymerization product was not detected. In Entry 3, 8% mol of $BF_3{\cdot}Et_2O$ was used in the polymerization set at -16°C for 2 days. This condition was similar to those reported by Sohn *et al.* [17], i.e., monomer concentration of 0.3 M, and initiator mole ratio (of 8% monomer). The result obtained was however, disappointing. No polymerization product was detected. This may be due to the difference in the

monomer structure hence different reaction condition (temperature, initiator) is required. In Entry 4, the temperature was raised to room temperature, resulting in 55% yield. Increase polymerization time to 5 and 7 days also helped increase the yield to 60 (Entry 5) and 76 % (Entry 6), respectively. Increased the amount of initiator from 1 to 8% resulted in an increase of yield (Entry 1, 4 and 8). However, at 24 %BF_3 the % yield did not significantly increase.

Additionally, the $SnCl_4$ was also used as an initiator in this study. The polymerization was carried out using 8% $SnCl_4$ at room temperature for 7 days. This initiator gave 50 % yield. Both BF_3 and $SnCl_4$ give products of similar structure.

3.2 Polymer Characterization

3.2.1 Molecular weight determination

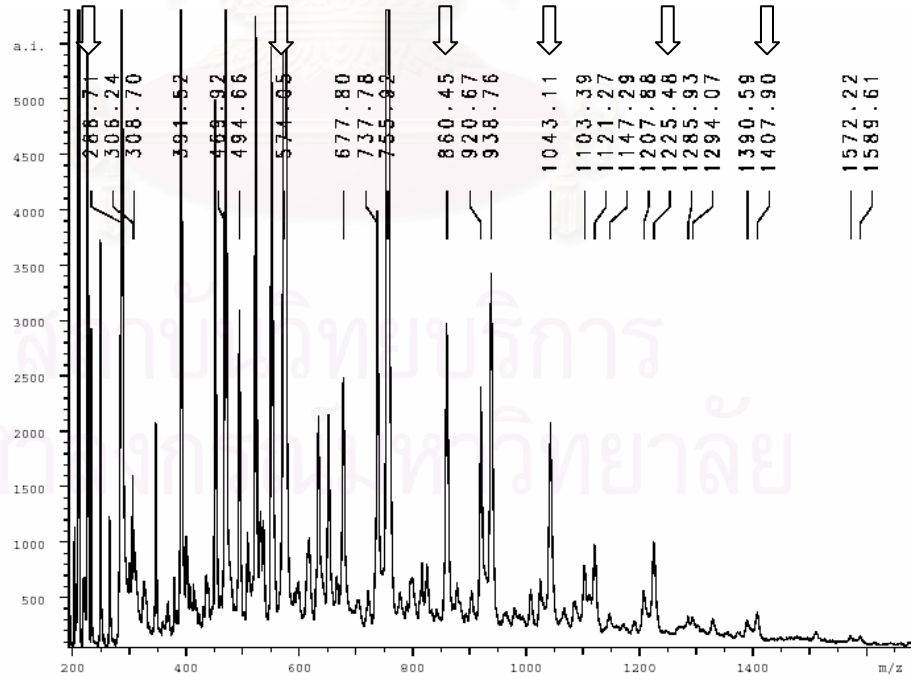


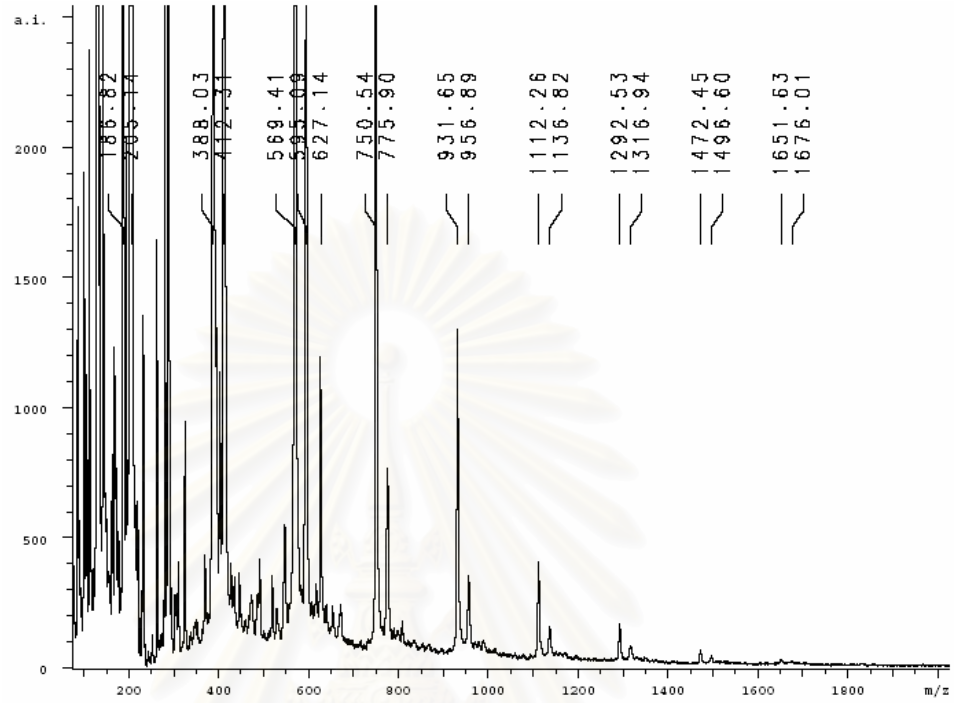
Figure 3.13 MALDI-TOF-MS of products after the polymerization of N^1-glycidyl-N^3-benzoylthymine

Molecular weights were measured by MALDI-TOF-MS. From Figure 3.13, the mass spectrum of benzoyl-protected polymer or poly(N^1-glycidyl-N^3-benzoylthymine) shows a number of signals. The mass of protected repeat unit is 286.29 and of the deprotected unit is 182.18. By considering the mass difference between signals, the peaks at 286.71, 574.05, 860.45, 1043.11, 1225.48 and 1407.90 can be interpreted into oligomer chains having randomly-distributed protected and deprotected repeat units. However, a number of unidentifiable peaks are also present.

It has to be kept in mind that this step, the expected reaction was the ring-opening of epoxide. But with the evidences from MS and previously shown NMR (Figure 3.6), it seems that partial removal of the benzoyl group took place. It is most likely that BF_3 also acts as a catalyst to hydrolyze the amide, thus releasing the benzoyl group. This is therefore one explanation for the low $\overline{M}_w$ obtained.

After deprotection of the benzoyl group in base, the mass spectrum of thymine oligomer (Figure 3.14) shows clusters of three intense peaks due to the protonated ($M\text{-}H^+$), sodiated ($M\text{-}Na^+$), and potassiated ($M\text{-}K^+$) quasi-molecular ions of the oligomers.

a) entry 2



b) entry 4

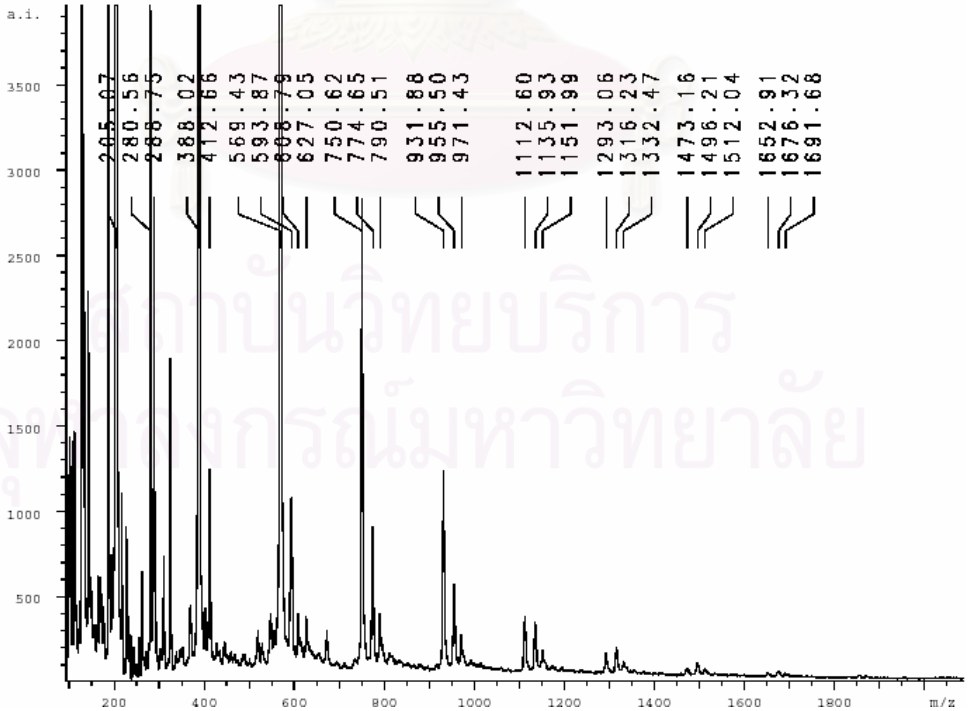
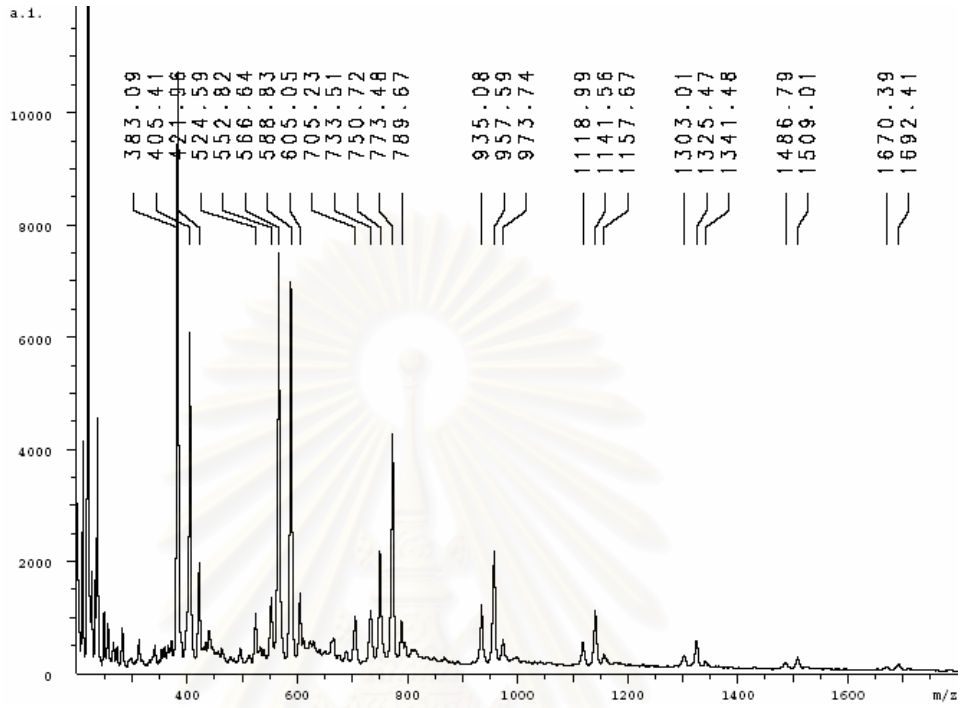


Figure 3.14 MALDI-TOF-MS of poly(N^1-glycidylthymine), a) entry 2 and b) entry 4. Entry 1 and 3 are not available for molecular weight determination.

c) entry 5



d) entry 6

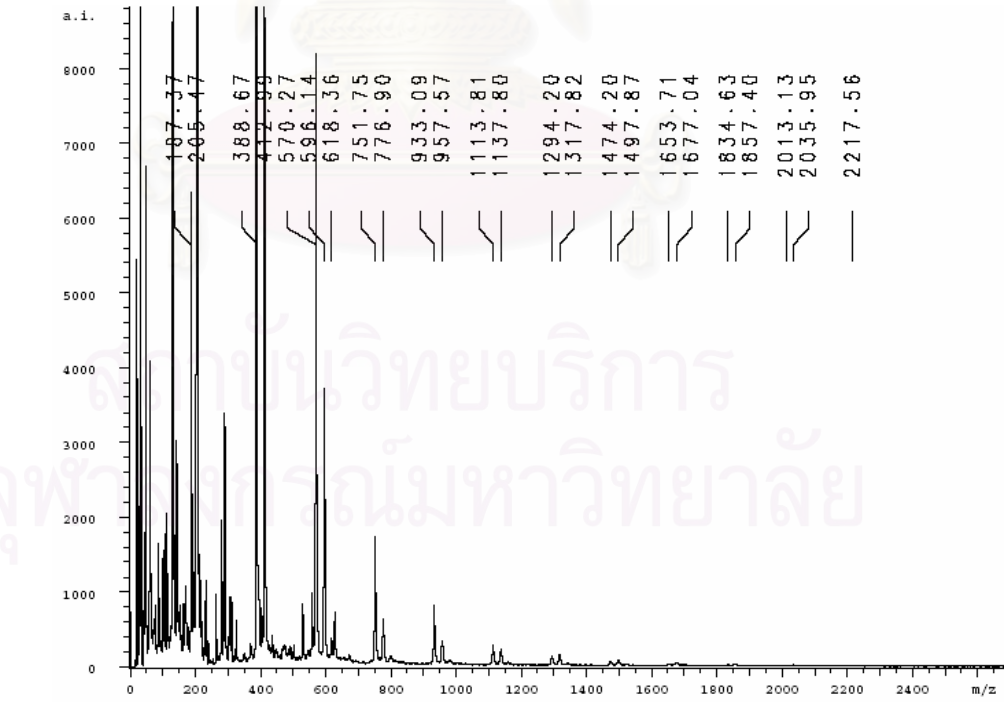
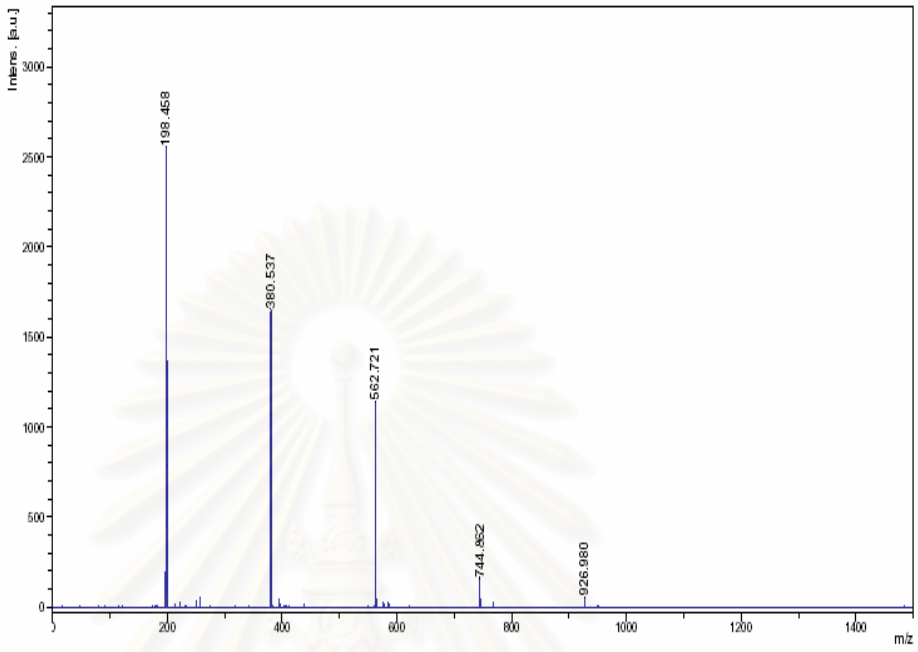


Figure 3.14 (Continued) MALDI-TOF-MS of poly(N^1-glycidylthymine), c) entry 5 and d) entry 6. Entry 1 and 3 are not available for molecular weight determination.

e) entry 7



f) entry 8

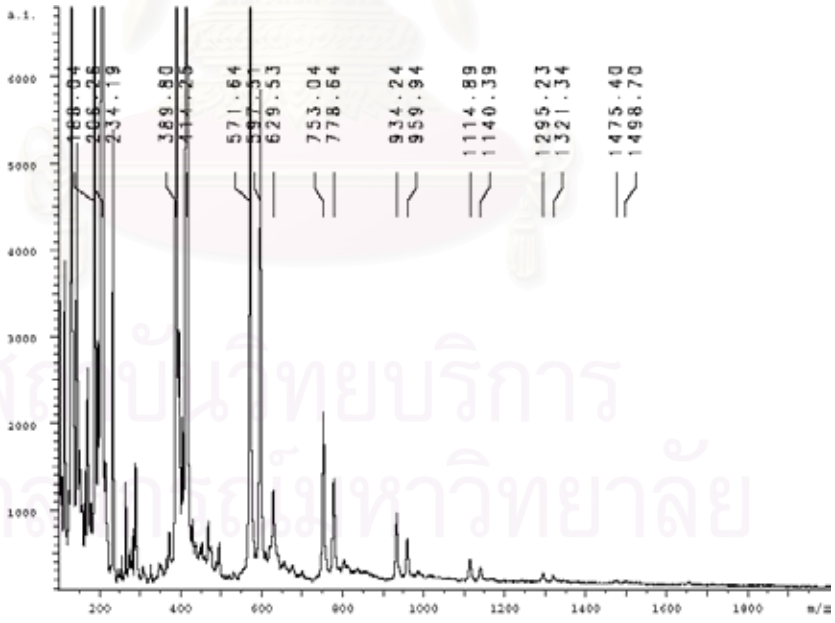


Figure 3.14 (Continued) MALDI-TOF-MS of poly(N^1-glycidylthymine), e) entry 7 and f) entry 8. Entry 1 and 3 are not available for molecular weight determination.

The average molecular weights of obtained products are listed in Table 3.6 When comparing $\overline{M}_w$ of the products in Entry 4, 5 and 6, increasing reaction time did not affect the $\overline{M}_w$.

Table 3.6 The average molecular weights of polymerization of N^1-glycidyl-N^3-benzoylthymine.

Entry	initiator		Temp.	Time	Thymine oligomer		
	type	%mol	(°C)	(days)	$\overline{M}_w$	$\overline{M}_n$	PDI
1	$BF_3{\cdot}Et_2O$	1	rt	7	N/A	N/A	N/A
2	$BF_3{\cdot}Et_2O$	4	rt	7	552	499	1.11
3	$BF_3{\cdot}Et_2O$	8	-16	2	N/A	N/A	N/A
4	$BF_3{\cdot}Et_2O$	8	rt	2	622	551	1.13
5	$BF_3{\cdot}Et_2O$	8	rt	5	655	556	1.18
6	$BF_3{\cdot}Et_2O$	8	rt	7	570	508	1.12
7	$BF_3{\cdot}Et_2O$	24	rt	7	413	334	1.24
8	$SnCl_4$	8	rt	7	531	603	1.14

N/A not available

Increase %mol of BF_3 also did not affect the $\overline{M}_w$ (Entry 2, 6 and 7). Theoretically, $\overline{M}_w$ should increase when decreasing the ratio of initiator to monomer. At 1% initiator concentration, the yield obtained is so low that further analysis of the product was impossible. From these observations, it is possible that the initiator is deactivated or consumed by the carbonyl groups in the monomer. BF_3 is a Lewis acid that is able to accept electrons from oxygen atom of the carbonyl.

The highest detectable molecular weight is 1,670 from Entry 5 (Figure 3.14 (c)). The weight-average molecular weight ($\overline{M}_w$) of 655 is equal to 3 repeat units (n=3). The low molecular weight product was obtained because the initiator was most

likely deactivated after binding to the carbonyl groups in the monomer. In addition, the repeat unit having benzoyl protecting group (mass=286) is not present in the mass spectrum. It is therefore confirmed that the benzoyl protecting group has been removed but the benzamide by-product remains in the polymer, as observed by the NMR analysis (Figure 3.8). Attempts to remove the benzamide by product by stirring the oligomer in ether could not, however, completely get rid of the benzamide.

The thymine oligomer obtained in entry 6 was use to further analysis.

3.2.2 End-group identification

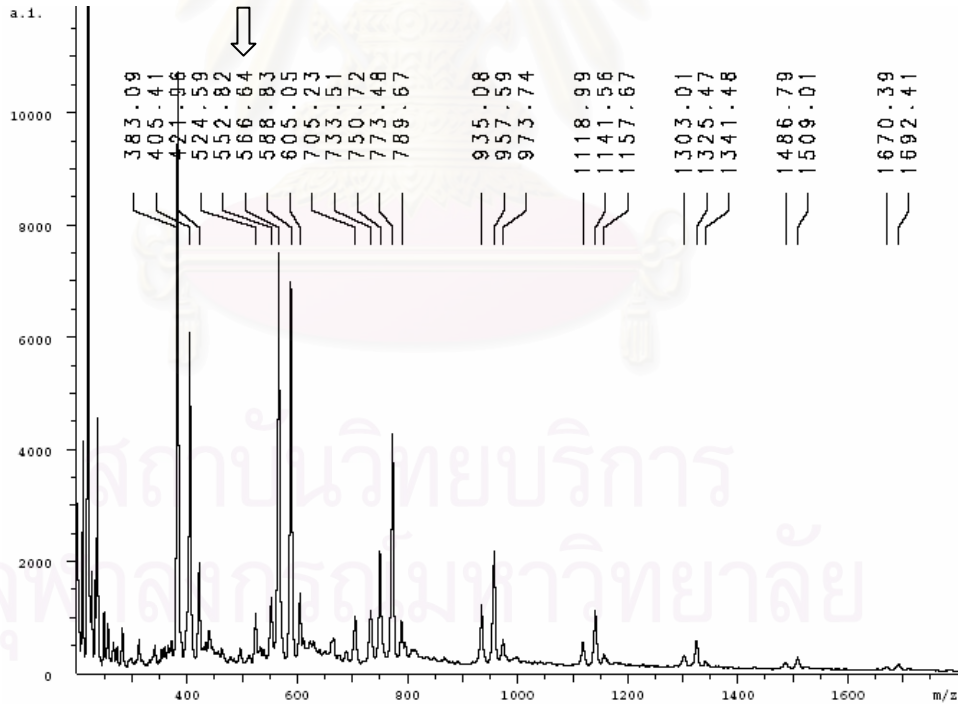


Figure 3.15 MALDI-TOF spectrum of entry 5 from Table 3.6

Consideration from the mechanism of polymerization of N^1-glycidyl-N^3-benzoylthymine in Scheme 3.5, the thymine oligomer should contain hydroxyl

chain end. From the MALDI-TOF-MS (Figure 3.15), a signal at 566 Da was observed. This corresponded to the molecular weight of three repeat unit with hydroxyl chain end.

3.2.3 Thermal analysis

3.2.3.1 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is a useful analytical technique for recording weight loss of a test sample as a function of temperature which may then be used for an understanding of the chemical nature of the polymer. TGA provides information about stability and decomposition of the material in an inert atmosphere [20].

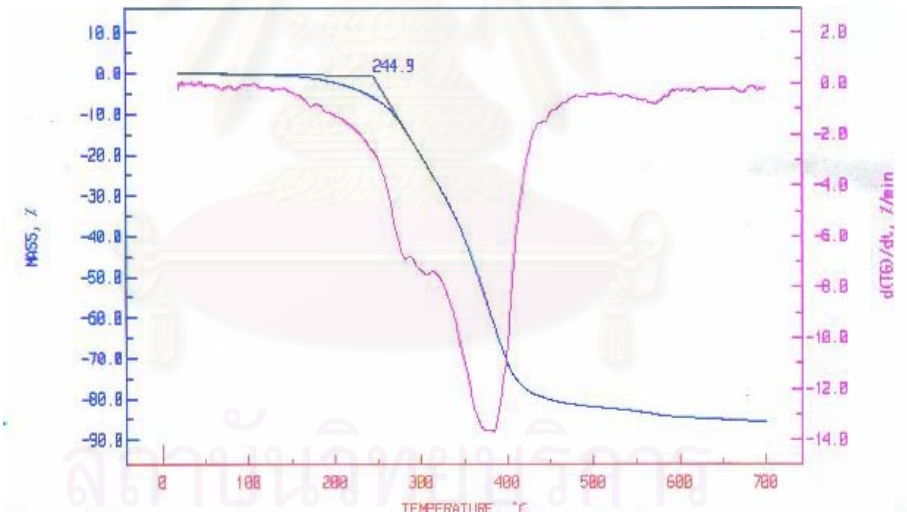


Figure 3.16 TGA of poly(N^1-glycidylthymine)

The decomposition temperature (T_d) of poly(N^1-glycidylthymine) is obtained at the on-set of decomposition which was found at 245°C (Figure 3.16). All chemical components of thymine oligomer are decomposed at 245°C. Therefore, thymine oligomer is not suitable for use at temperature higher than 245°C.

3.2.3.2 Differential Scanning Calorimetry (DSC)

Glass transition temperatures (T_g) are most commonly measured by differential scanning calorimetry (DSC). In DSC, a polymer sample and an inert reference are heated, usually in a nitrogen atmosphere, and thermal transitions in the sample are detected and measured [18].

T_g of 36 °C is obtained from the inflection point of the thermogram (Figure 3.17). Below 36 °C thymine oligomer is in the glass state, at 36 °C it is changing from glass to rubbery state, and beyond 36 °C it continues in the rubbery state.

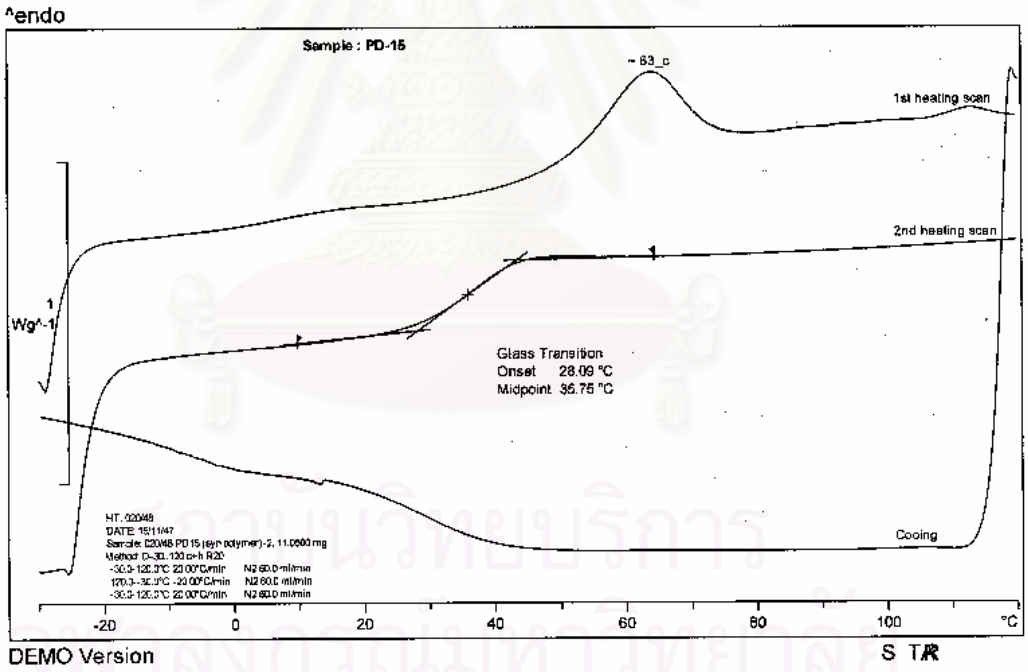


Figure 3.17 DSC analysis of poly(N^1-glycidylthymine)

3.2.4 UV spectroscopy

From Figure 3.18, the λ_{max} of thymine oligomer was at 273 nm, close to that thymine (at 266 nm), indicating that the obtained product contained thymine unit. The λ_{max} of N^1-glycidyl-N^3-benzoylthymine was at 253 nm, far away from the thymine oligomer.

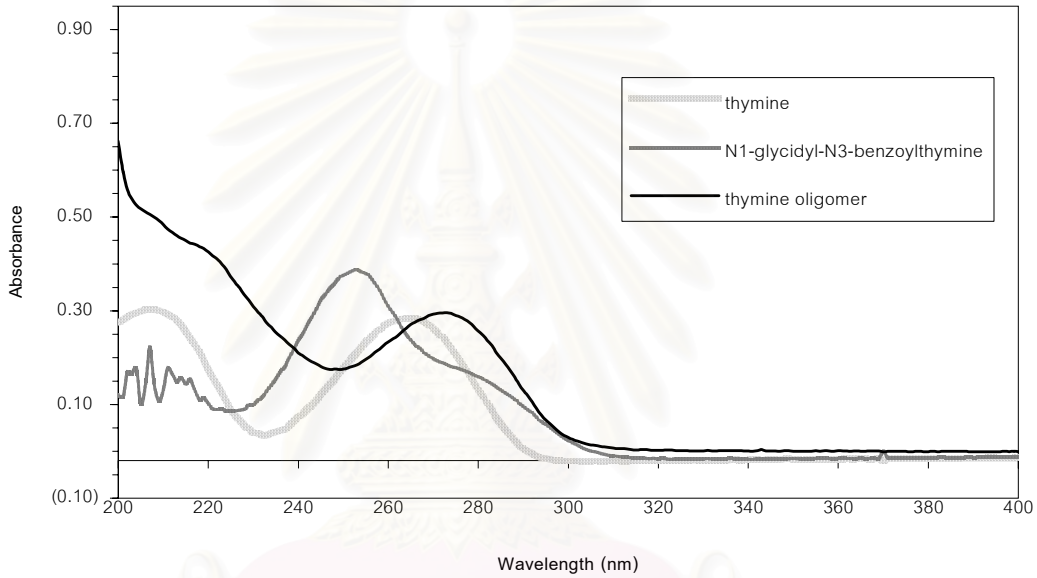


Figure 3.18 UV spectrums of thymine, N^1-glycidyl-N^3-benzoylthymine and poly(N^1-glycidylthymine)

3.2.5 Interaction of poly(N^1-glycidylthymine) with nucleic acid

The melting temperature (T_m) is defined as the temperature at which 50% of the DNA exists as a duplex and 50% are single stranded [25]. Here poly(N^1-glycidylthymine) was tested for the interaction with DNA and RNA by UV spectroscopy method. If the binding between poly(N^1-glycidylthymine) and polynucleotide occurs, a transition in the absorbance spectrum will be observed. The results are shown in Figure 3.19. In all cases the experiment was carried out on a

cooling/reheating cycle. No binding between poly(N^1-glycidylthymine) with poly(rA) or poly(dA) was observed. This could be due to many possible reasons. First, it may be possible that the molecular weight of poly(N^1-glycidylthymine) was too low to bind with its complimentary base pair (A). The length of the oligomer was not sufficient to produce strong binding. Second, the space between each thymine nucleobase was shorter than that in the nucleic acid. Last, the structural backbone of poly(N^1-glycidylthymine) is more flexible than polynucleotide. Due to, the base pairing of two strands occurs when the two bases are in the suitable conformation. The limiting conformation leads to entropy loss. So, the flexible polymer hardly locks its conformation to pair with polynucleotide.

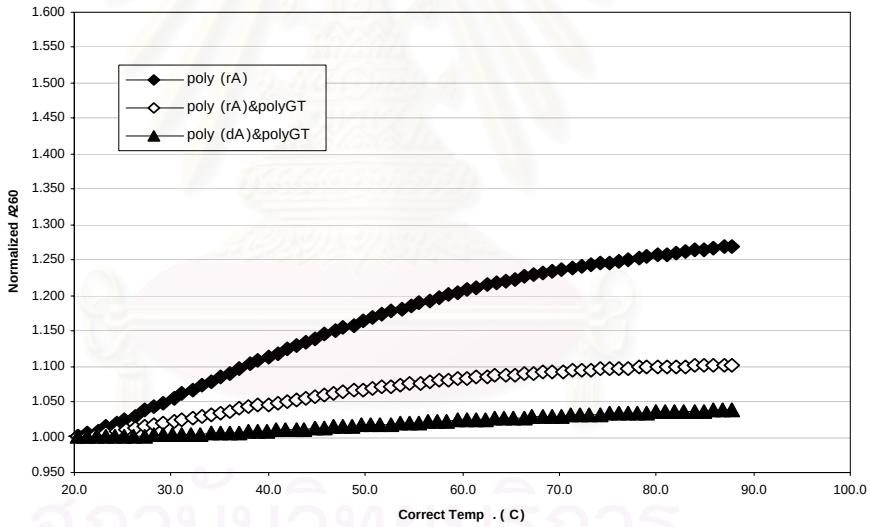


Figure 3.19 Melting curve of 1:1 mixture between poly(N^1-glycidylthymine) and poly(rA) and poly(dA) in 100 mM Sodium phosphate buffer, pH 7.0

CHAPTER IV

CONCLUSION

A novel oligomer with thymine in every repeat unit was synthesized. First, N^1-glycidyl-N^3-benzoylthymine monomer was synthesized by two trial methods. In the first method N^3-benzoylthymine was reacted with glycidol by Mitsunobu reaction pathway. In the second method, the monomer was synthesized from epoxidation of N^1-allyl-N^3-benzolythymine. Comparing between two methods, the Mitsunobu reaction gave higher yield than the epoxidation pathway. Therefore, the first method was chosen for the synthesis of N^1-glycidyl-N^3-benzoylthymine monomer.

The CROP was carried out in the presence of $BF_3 \cdot Et_2O$ as initiator. During the polymerization some benzoyl protecting groups were removed. Complete removal of the benzoyl protecting group was carried out in basic condition but the benzamide by-product remains absorbed in the polymer.

Initiator concentration did not affect the weight-average molecular weight. The maximum detectable molecular weight was 1,670 Da and the $\overline{M}_w$ was about 600 Da. The low molecular weight product was obtained because the initiator was most likely deactivated after binding to the carbonyl groups in the monomer.

The UV absorbance of the product indicated that the obtained product contained thymine unit. Its interactions with poly(deoxyadenylic acid) and poly(adenylic acid) were, however, not observed.

Proposal to the future work

The benzoyl protecting group interrupted the polymerization in the presence of BF_3. A change of the protecting group to benzyl instead of benzoyl could be a better method for the polymerization using a Lewis acid initiator.

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APPENDIX

APPENDIX A

Table A-1 Molecular weight determination (Table 3.6, entry 2)

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
388.78	1.51×10^5	1.24×10^4	4.81×10^6	1.87×10^9
570.58	3.26×10^5	7.20×10^3	4.11×10^6	2.34×10^9
751.94	5.65×10^5	1.44×10^3	1.08×10^6	8.15×10^8
933.28	8.71×10^5	5.34×10^2	4.98×10^5	4.65×10^8
1114.13	1.24×10^6	1.77×10^2	1.97×10^5	2.20×10^8
1294.86	1.68×10^6	8.44×10^1	1.09×10^5	1.41×10^8
1474.41	2.17×10^6	4.14×10^1	6.11×10^4	9.01×10^7
1653.82	2.74×10^6	3.04×10^1	5.03×10^4	8.32×10^7
		$\sum n_i = 2.19\times10^4$	$\sum m_i n_i = 1.09\times10^7$	$\sum m_i^2 n_i = 6.03\times10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i} \qquad \overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_w = \frac{6.03\times10^9}{1.09\times10^7} \qquad \overline{M}_n = \frac{1.09\times10^7}{2.19\times10^4}$$

$$\overline{M}_w = 552 \qquad \overline{M}_n = 499$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

$$PDI = \frac{552}{499}$$

$$PDI = 1.11$$

Table A-2 Molecular weight determination (Table 3.6, entry 4)

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
388.02	1.51×10^5	1.02×10^4	3.95×10^6	1.53×10^9
569.43	3.24×10^5	8.56×10^3	4.87×10^6	2.77×10^9
750.62	5.63×10^5	2.84×10^3	2.13×10^6	1.60×10^9
931.88	8.68×10^5	1.24×10^3	1.16×10^6	1.08×10^9
1112.60	1.24×10^6	3.82×10^2	4.25×10^5	4.73×10^8
1293.06	1.67×10^6	1.68×10^2	2.17×10^5	2.81×10^8
1473.16	2.17×10^6	8.27×10^1	1.22×10^5	1.79×10^8
1652.91	2.73×10^6	5.12×10^1	8.47×10^4	1.40×10^8
		$\sum n_i = 2.35\times10^4$	$\sum m_i n_i = 1.30\times10^7$	$\sum m_i^2 n_i = 8.06\times10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i} \qquad \overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_w = \frac{8.06\times10^9}{1.30\times10^7} \qquad \overline{M}_n = \frac{1.30\times10^7}{2.35\times10^4}$$

$$\overline{M}_w = 622 \qquad \overline{M}_n = 551$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

$$PDI = \frac{622}{551}$$

$$PDI = 1.13$$

Table A-3 Molecular weight determination (Table 3.6, entry 5)

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
383.09	1.47×10^5	1.07×10^4	4.11×10^6	1.57×10^9
566.64	3.21×10^5	7.50×10^3	4.25×10^6	2.41×10^9
750.72	5.64×10^5	2.19×10^3	1.64×10^6	1.23×10^9
935.08	8.74×10^5	1.22×10^3	1.14×10^6	1.07×10^9
1118.99	1.25×10^6	5.64×10^2	6.31×10^5	7.06×10^8
1303.01	1.70×10^6	3.27×10^2	4.26×10^5	5.55×10^8
1486.79	2.21×10^6	1.95×10^2	2.90×10^5	4.31×10^8
1670.39	2.79×10^6	1.24×10^2	2.07×10^5	3.45×10^8
		$\sum n_i = 2.28\times10^4$	$\sum m_i n_i = 1.27\times10^7$	$\sum m_i^2 n_i = 8.32\times10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i} \qquad \overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_w = \frac{8.32\times10^9}{1.27\times10^7} \qquad \overline{M}_n = \frac{1.30\times10^7}{2.35\times10^4}$$

$$\overline{M}_w = 655 \qquad \overline{M}_n = 556$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

$$PDI = \frac{655}{556}$$

$$PDI = 1.18$$

Table A-4 Molecular weight determination (Table 3.6, entry 6)

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
388.67	1.51×10^5	1.43×10^4	5.54×10^6	2.15×10^9
570.27	3.25×10^5	8.20×10^3	4.67×10^6	2.67×10^9
751.75	5.65×10^5	1.73×10^3	1.30×10^6	9.79×10^8
933.09	8.71×10^5	8.26×10^2	7.70×10^5	7.19×10^8
1113.81	1.24×10^6	2.96×10^2	3.29×10^5	3.67×10^8
1294.20	1.67×10^6	1.49×10^2	1.93×10^5	2.49×10^8
1474.20	2.17×10^6	7.25×10^1	1.07×10^5	1.58×10^8
1653.71	2.73×10^6	3.84×10^1	6.36×10^4	1.05×10^8
		$\sum n_i = 2.56\times10^4$	$\sum m_i n_i = 1.30\times10^7$	$\sum m_i^2 n_i = 7.39\times10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i} \qquad \overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_w = \frac{8.06\times10^9}{1.30\times10^7} \qquad \overline{M}_n = \frac{1.30\times10^7}{2.35\times10^4}$$

$$\overline{M}_w = 570 \qquad \overline{M}_n = 508$$

$$\text{PDI} = \frac{\overline{M}_w}{\overline{M}_n}$$

$$\text{PDI} = \frac{570}{508}$$

$$\text{PDI} = 1.12$$

Table A-5 Molecular weight determination (Table 3.6, entry 7)

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
198.45	3.94×10^4	2.38×10^3	4.73×10^5	9.38×10^7
380.52	1.45×10^5	2.08×10^3	7.92×10^5	3.01×10^8
562.69	3.17×10^5	1.82×10^3	1.02×10^6	5.77×10^8
744.83	5.55×10^5	2.64×10^2	1.97×10^5	1.46×10^8
926.96	8.59×10^5	1.01×10^2	9.36×10^4	8.68×10^7
		$\sum n_i = 6.65\times10^3$	$\sum m_i n_i = 2.58\times10^6$	$\sum m_i^2 n_i = 1.20\times10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i} \qquad \overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_w = \frac{1.20\times10^9}{2.58\times10^6} \qquad \overline{M}_n = \frac{2.58\times10^6}{6.65\times10^3}$$

$$\overline{M}_w = 467 \qquad \overline{M}_n = 388$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

$$PDI = \frac{467}{388}$$

$$PDI = 1.20$$

Table A-6 Molecular weight determination (Table 3.6, entry 8)

m_i	m_i^2	n_i	$m_i n_i$	$m_i^2 n_i$
389.80	1.52×10^5	1.35×10^4	5.24×10^6	2.04×10^9
571.64	3.27×10^5	9.58×10^3	5.48×10^6	3.13×10^9
753.04	5.67×10^5	2.13×10^3	1.61×10^6	1.21×10^9
934.24	8.73×10^5	9.70×10^2	9.06×10^5	8.47×10^8
1114.89	1.24×10^6	4.36×10^2	4.87×10^5	5.42×10^8
1295.23	1.68×10^6	2.79×10^2	3.62×10^5	4.69×10^8
1475.40	2.18×10^6	1.97×10^2	2.91×10^5	4.29×10^8
		$\sum n_i = 2.71\times10^4$	$\sum m_i n_i = 1.44\times10^7$	$\sum m_i^2 n_i = 8.67\times10^9$

$$\overline{M}_w = \frac{\sum m_i^2 n_i}{\sum m_i n_i} \qquad \overline{M}_n = \frac{\sum m_i n_i}{\sum n_i}$$

$$\overline{M}_w = \frac{8.67\times10^9}{1.44\times10^7} \qquad \overline{M}_n = \frac{1.44\times10^7}{2.71\times10^4}$$

$$\overline{M}_w = 603 \qquad \overline{M}_n = 531$$

$$PDI = \frac{\overline{M}_w}{\overline{M}_n}$$

$$PDI = \frac{603}{531}$$

$$PDI = 1.14$$

VITAE

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