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Preparation of novel amphiphilic polymers via ring-opening metathesis polymerization and the study of their antibacterial properties.

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PREPARATION OF NOVEL AMPHIPHILIC POLYMERS VIA RING-OPENING METATHESIS POLYMERIZATION AND STUDY OF THEIR ANTIBACTERIAL PROPERTIES

A Dissertation Presented

by

MEHMET FIRAT ILKER

Submitted to the Graduate School of the
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Polymer Science and Engineering

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DEDICATION

To my beloved parents Şeyma and Kutal Ilker, my dear brother Onur Ilker,
Ilker family, and my grandfather Mehmet Emin Ilker

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ABSTRACT

PREPARATION OF NOVEL AMPHIPHILIC POLYMERS VIA RING-OPENING METATHESIS POLYMERIZATION AND STUDY OF THEIR ANTIBACTERIAL PROPERTIES

FEBRUARY 2005

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This thesis adapted tools of organic and organometallic chemistry to achieve control over synthetic macromolecular architectures, with a focus on the systematic incorporation of polar and nonpolar chemical entities into polymers, and test these amphiphilic polymers for their interactions with living cells, bacterial and mammalian.

The development of highly active well-defined catalyst systems for olefin metathesis, and their influence on the development of ring-opening metathesis polymerization (ROMP) has been a major inspiration behind our synthetic strategy towards the preparation of model amphiphilic polymer architectures with a high level of structural control. The first synthetic approach was the investigation of ring-opening metathesis copolymerization of polar and nonpolar cyclic olefins as monomers. This study leads to the discovery of alternating copolymerizations of a series of polar cyclic olefins with nonpolar cyclic olefins using ruthenium-based homogeneous catalyst system. Mechanistic studies revealed that steric factors induced from comonomer structures and catalyst type affect the degree of alternation on the polymer backbone.

This novel technique allows for the strictly alternating incorporation of polar and nonpolar monomeric units into polymer chains of various lengths, and facilitates the polymerization of sterically encumbered monomers and modification of final material properties.

In a second synthetic approach, a general strategy was developed for the assembly of polar and nonpolar domains into a modular monomer structure. The character and size of each domain can be tuned independently and locked into the repeating unit of the amphiphilic polymers resulting from ROMP of the modular norbornene derivatives. Living ROMP of these monomers provided access to a large range of molecular weights with narrow molecular weight distributions.

Lipid membrane disruption activities, a key feature of amphiphilic polymers used in many biomedical applications, were investigated for amphiphilic polynorbornene derivatives against liposomes. Water-soluble amphiphilic cationic polynorbornene derivatives, which exhibited the highest level of activities against liposome membranes, were then probed for their antibacterial activities in growth inhibition assays and hemolytic activities against human red blood cells in order to determine the selectivity of the polymers for bacterial over mammalian cells. By tuning the overall hydrophobicity of the polymer, highly selective, non-hemolytic antibacterial activities were obtained.

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INTRODUCTION

1.1 Functional Polymers

From research laboratories to large scale industries polymeric materials have found, and continue to find, applications in very diverse fields, as cement additives in civil engineering[1] or drug delivery vehicles in medical science.[2] While polymers have been shown to share common material properties resulting from their macromolecular nature, the diversity on the chemical character results in a wide range of properties. Polymer science, as a highly branched interdisciplinary science, has improved through the understanding of structure property relationships, which allows for the design of synthetic polymers and targets a variety of applications. Through this scientific evolution, control over macromolecular architecture and resulting material properties has been a central goal of polymer chemistry. The presence of any functional group on the repeating unit of a polymer not only dictates its self association and related material properties (e.g. melting temperature, glass transition temperature) but also its interactions with its environment.[3,4] Those interactions can be very simple such as solubility, adhesion onto a substrate or very complex such as the biochemical activities of highly functionalized natural polymers (e.g. DNA, proteins).

Introducing polar and/or complex chemical functionalities has become increasingly important as polymer chemists start to target properties such as bioactivity or mimic the function of biopolymers. The highly reactive nature of propagating species in conventional chain growth polymerizations, such as radicals and anions, brings limitations to the use of these synthetic techniques for incorporation of polar

functional groups. Step-growth polymerization, on the other hand, lacks the control over molecular weight, an important issue in preparing specialty polymers with well-controlled properties. In order to overcome synthetic obstacles associated with conventional polymer synthesis techniques efforts have been directed towards the evaluation of new methodologies whereby precise placement of a desired chemical functionality into polymer structure can be achieved. Such efforts are commonly stimulated by new developments in organic, inorganic, and organometallic chemistries. Ring-opening metathesis polymerization (ROMP), based on olefin metathesis, has attracted considerable research attention recently in large part due to development of well-defined catalyst systems that provides broad flexibility over the choice of functional groups on the monomer unit and a high level of control over the macromolecular architecture.[5,6] This dissertation will present ROMP as the central synthetic tool to be used for the preparation of well-defined polymer architectures bearing multiple functional groups.

1.2 Olefin Metathesis and Ring-Opening Metathesis Polymerization

Olefin metathesis, the metal catalyzed redistribution of carbon-carbon double bonds, is currently undergoing an exciting evolution due to the recent progress in developing homogeneous catalyst systems and their wide-spread use in organic and polymer synthesis.[7] Possible applications include not only ROMP but also, ring-closing metathesis (RCM), acyclic diene metathesis polymerization (ADMET) (Figure 1.1), ring-opening metathesis (ROM) (Figure 1.2), and cross-metathesis (CM or XMET) (Figure 1.3).[7] Historically, the most common application of olefin metathesis has been the preparation of new polymeric materials through ROMP.[7] Highly active metathesis

catalysts based on group 6 metals, in particular molybdenum, have been developed by Schrock (Figure 1.4, **1**).[8-10] These catalysts allow for the living ROMP of a variety of monomers and provide control over polymer microstructure such as cis/trans ratios and tacticity.

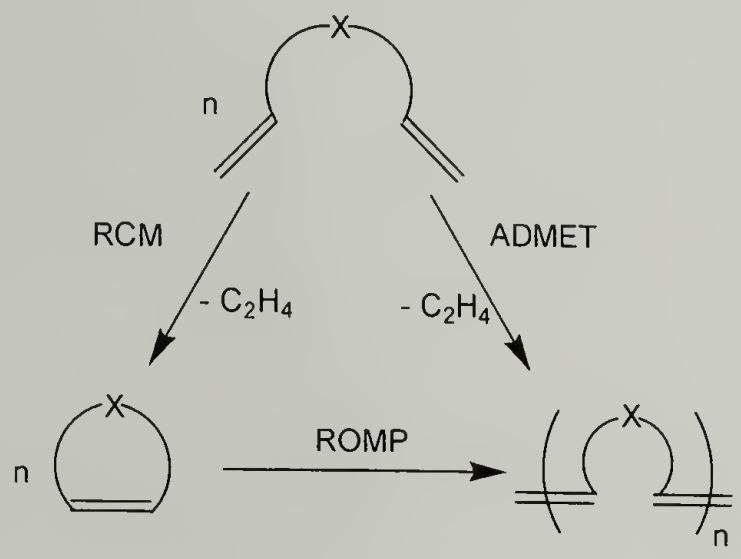


Figure 1.1 Ring opening metathesis polymerization (ROMP) of cyclic olefins, ring closing metathesis (RCM) of acyclic dienes, and acyclic diene metathesis (ADMET) polymerization.[7]

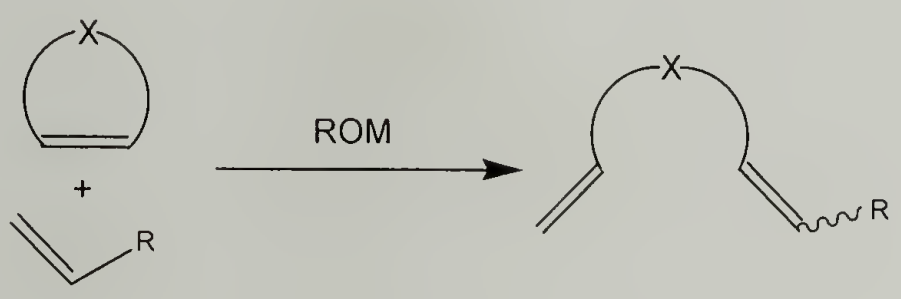


Figure 1.2 Ring opening metathesis reaction of cyclic olefins with acyclic olefins.

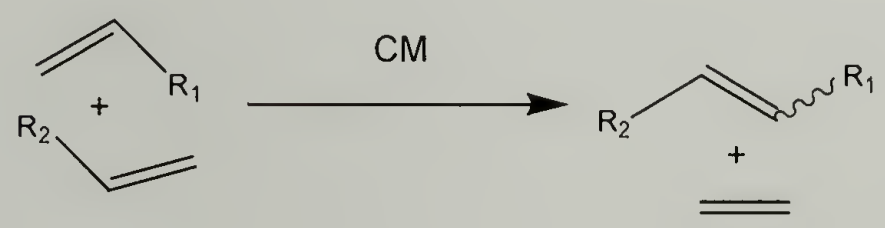
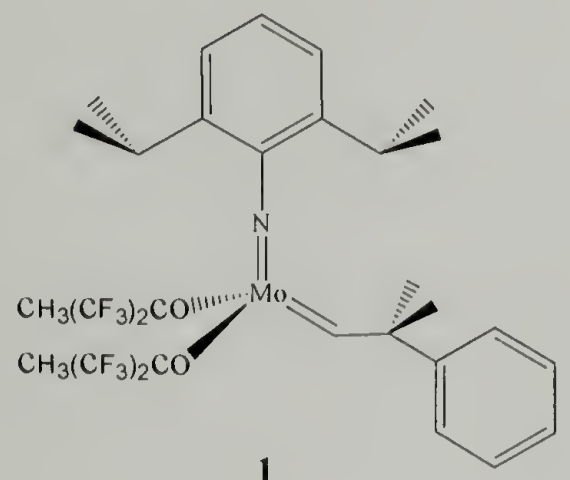


Figure 1.3 Cross-metathesis reaction of two acyclic olefins.

More recently, the ruthenium-based catalyst systems introduced by Grubbs permit metathesis reactions in polar and nonpolar reaction media in addition to being tolerant towards a range of protic and polar functional groups under ambient conditions (Figure 1.4, **2-4**).[7] These commercially available and relatively inexpensive catalyst systems have been the major factor that helped ROMP become a powerful and commonly used polymer synthesis technique.



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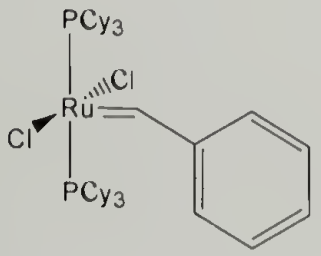

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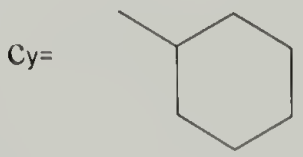

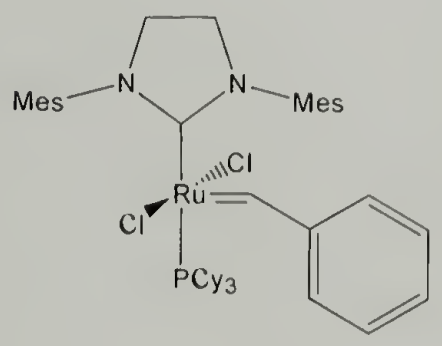

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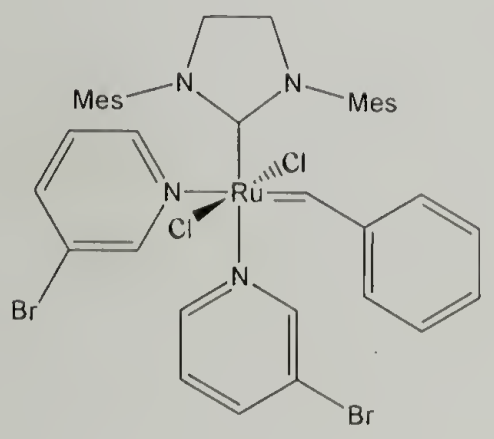

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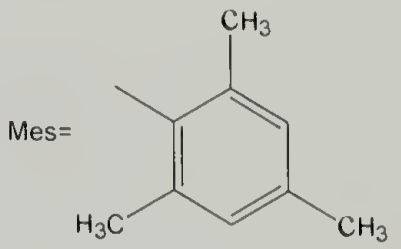

Figure 1.4 Catalysts **1** (Schrock catalyst), **2** (Grubbs catalyst), **3** (second generation Grubbs catalyst) and **4** (Grubbs-Love catalyst).

1.3 Functionalized Cyclic Olefins as monomers for ROMP

It is most often the introduction of a new monomer, rather than a new polymerization technique, that yields novel synthetic polymer structures. Modifications over the side group functionality of the monomer structure, rather than its polymerization site, is commonly used to dictate the resulting material properties.[11] Functional group tolerance and high activity of homogeneous catalyst systems allowed

for the sereening of a large number of monomers, with numerous funetional groups, for ROMP. Conversely, the availability of inexpensive eyelie olefins as monomers (Figure 1.5), and the facile synthetie aeeess to funetionalized eyelie olefin derivatives stimulatcd a large body of researeh on ROMP.[12,13]

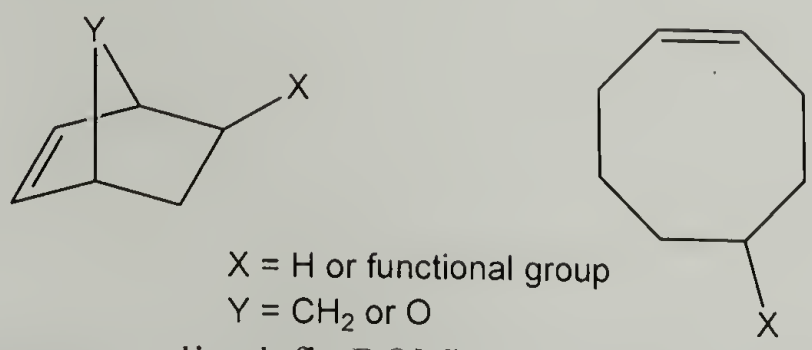

Figure 1.5 Common eyclie olefin ROMP monomers. Norbornene derivatives (left), cyclooctene derivatives (right).

The variety of novel polymer compositions that were prepared via ROMP in the last ten ycars is unmatched by any other polymcrization teehnique. Notable examples includc: Block copolymcrs,[14,15] fluoropolymers,[16] high-temperature polymers,[17] hydrogels,[18] polyelcctrolytes,[19,20] side chain liquid crystal polymers,[21] and polymers functionalized with biologically relevant sidc groups.[22] The latter group of polymers has reached a rcmarkable levcl of divcrsity including polymers that are functionalizcd by oligopeptides,[23] oligonucleotides,[24] carbohydrates,[25] and anti-eancer drugs.[26] These ROMP-based synthetic dcvelopmcnts as a wholc, allow this technique to beeome an important toolbox for thc design of biologically activc polymcrs, which can potentially mimic the complex aetivities of natural macromolecules.

1.4 Synthetic Strategy

Thc cxamplcs listed above successfully demonstrate the compatibility of ruthenium-bascd catalyst systems with various functional groups. However therc are intrinsic disadvantages of polar and/or largc functional groups on thc monomer unit.

The excess steric and polar interactions limit the molecular weight build up during polymerization and possibly result in poor polymer properties such as low solubility in organic solvents. The presence of both polar and nonpolar character in a monomer are expected to provide strong assets to polymeric material. Hydrocarbon based nonpolar domains are generally considered to be an ideal structural component providing chemical inertness and processability, where the presence of polar functionality is expected to allow improved interactions with target substrate. With this vision, the initial target of this dissertation has been the use of metathesis polymerization as a versatile technique to introduce and tune the balance of both polar and nonpolar functionalities into well-defined polymers. The ROMP-based synthetic approach in this body of work can be divided into two main sections. The first approach is the copolymerization of polar and nonpolar cyclic olefins.[27] The relatively new ruthenium-based ROMP literature has rather limited examples of copolymerization, which conventionally is a powerful synthetic approach to tune final polymer properties.[28] Therefore the first portion of this thesis focused on the understanding of mechanistic aspects of ring-opening metathesis copolymerization based on commonly used homogeneous catalyst systems. The effects of comonomer, and catalyst choice on the degree of control over copolymerization, from random to perfectly alternating copolymerization, was determined and will be presented. The resulting novel polymer structures contain desired polar functional groups regularly separated by hydrophobic spacers along the backbone. This technique not only gave access to a class of well-defined amphiphilic copolymers but also facilitates ROMP of monomers bearing bulky or highly polar substituents.

The second approach was the preparation and ROMP of a novel class of monomers bearing dual functionality.[29] Modular norbornene derivatives are designed to contain two separate domains in close proximity, where the nature and balance of polar and nonpolar functionalities are tuned and locked into the monomer structure. Extending the polar character into a water soluble functionality, allows this technique to become a novel approach for the preparation of well-defined amphiphilic polymers. In general the amphiphilicity of a polymer arises from the amphiphilic nature of a single substituent on each repeating unit without a good control over the hydrophilic/hydrophobic balance. The hydrophilic/hydrophobic balance and the character of the polar functionality are key structural features that dictates the interactions of an amphiphilic polymer with its environment (biogenic or abiogenic). The objective of modular norbornene design is to offer a facile route to control basic structural features of an amphiphilic polymer and to probe its behavior.

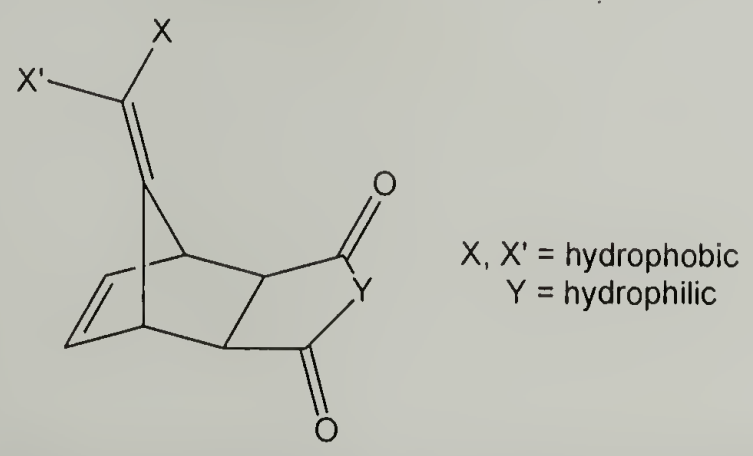

Figure 1.6 General structure of amphiphilic modular norbornene derivative.

1.5 Applications for Well-Defined Amphiphilic Polymers: Antibacterial Activity

Well-defined amphiphilic macromolecules find important applications in biology and medical sciences. Examples include the use of polymeric materials in drug delivery,[30-34] gene delivery,[35-38] tissue engineering,[39-41] antibiotic agent applications.[42-48] Continuing research efforts are focusing on the use of polymeric therapeutics as

alternative antibiotic agents in the fight against bacterial diseases. Antibacterial activity of cationic polymers have been known for several decades.[43] Various polymeric structures carrying cationic moieties have found considerable interest in non-medical use such as food preservatives, pesticides, and disinfectants.[42] Very recently antibacterial activity of relatively simple cationic polymers has started to be considered within the scope of the studies involving naturally occurring host-defense peptides, and their synthetic mimics.[49-51] Although more complex in their structure, antimicrobial peptides commonly contain cationic and hydrophobic domains.[52] Successful research efforts that target synthetic mimics of host-defense peptides has typically followed a top-down approach, through structural modifications of naturally occurring peptide structures, in an effort to establish an understanding of structure-property relationships.[53] In the development stage synthetic mimics of host-defense peptides require elaborate and extensive techniques.[54-57] Relatively simple synthetic cationic polymers offer an inexpensive alternative, however they suffer from their high cytotoxicity if considered for therapeutic applications.[42] Encouraged by the synthetic abilities for the controlled preparation of amphiphilic polymers, and inspired both by antimicrobial peptide research and synthetic biocidal polymers, this thesis work seeks a determination of macromolecular properties that allows for antibacterial activity while suppressing cytotoxicity. ROMP of amphiphilic modular norbornene derivatives allowed for the facile probing of the effect of basic macromolecular variables on the interactions of polymers with living cells, bacterial and mammalian.

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CHAPTER 2

ALTERNATING COPOLYMERIZATIONS OF POLAR AND NONPOLAR CYCLIC OLEFINS BY RING-OPENING METATHESIS POLYMERIZATION

2.1 Introduction

Large numbers of functional polymer architectures accessible via ROMP consist of homopolymers or block copolymers prepared by sequential monomer addition. The properties of these polymers are tuned by modifying the functionality on each monomer unit. Copolymerization, in general, provides a new route to tune material properties through combinations of various monomers and reaction stoichiometry. The study of copolymerization by ring-opening metathesis has not attracted much attention when compared to the corresponding homopolymerization.[1,2] The study of copolymerizations of a variety of easily accessible cyclic olefins by ring-opening metathesis holds promise for the preparation of versatile polymers with multiple functional groups (e.g. amphiphilic polymers). The understanding and control over the copolymerization and placement of different monomers in a polymer chain requires the study of copolymerization rates of a series of model cyclic olefin monomers. Depending on the choice of comonomers, copolymerization may result in a random copolymer, block or tapered block copolymer, or alternating copolymer microstructure. A special case of copolymerization, alternating copolymerization results in a well-defined polymer microstructure where the repeating unit consists of two comonomer units. These can possibly carry two different functionality, regularly placed in alternation.

Alternating copolymers can be synthesized by various polymerization methods.[3] However, alternating copolymerization of monomer mixtures by ring-opening metathesis polymerization is very rare. There have been only two reports in the

literature. The first report was the alternating copolymerization of the enantiomers of 1-methylnorbornene catalyzed by $ReCl_5$, in which it was not possible to polymerize an optically pure monomer due to steric effects.[4] The low activity of this heterogeneous catalyst and consequently its intolerance towards steric hinderence was presumably a key parameter in this alternation mechanism. The second example was the alternating copolymerization of cyclopentene and norbornene, two nonpolar monomers, using $RuCl_3$, $IrCl_3$ or $OsCl_3$ in the presence of phenol as a co-catalyst or solvent. A hydrogen-bonded solvent cage around the catalyst site was invoked to explain rapid cross-propagation relative to homopolymerization. The alternating distribution was obtained under condition of a 1:8 norbornene to cyclopentene feed ratio and was maintained throughout yields ranging from 2 to 20%.[5,6]

The synthetic utility of alternating ring-opening metathesis copolymerization can be expanded considering the recent progress in olefin metathesis that utilize highly active well-defined catalyst systems for polymerizations of various functionalized polar or nonpolar monomers. This chapter presents the first example of alternating ring-opening metathesis copolymerization that utilizes a homogeneous catalyst system and incorporates polar and nonpolar monomers resulting in a series of alternating copolymers with tailorable functionalities.[7]

2.2 Experimental Section

2.2.1 Materials

$Mo(CHCMe_2Ph)(NAr)(OCMe(CF_3)_2)$ (**1**), $RuCl_2(=CHPh)(PCy_3)_2$ (**2**), and (tricylohexylphosphine)(1,3-dimesitylimidazolidine-2-ylidine)benzylideneruthenium dichloride (**3**) were purchased from Strem Chemical. The $[(H_2Imes)(3\text{-Br-py})_2\text{-}(Cl)_2Ru{=}CHPh]$ (**4**)[8] and monomers *exo* **6**, and *exo* **7**[9] were prepared according to

literature procedures. All other reagents were obtained from Aldrich. Cyclooctene, cyclooctadiene, cyclopentene and deuterated chloroform were passed through columns of basic activated alumina prior to use. Methylene chloride was vacuum-distilled from CaH_2 prior to use. Norbornene was used as received.

2.2.2 Preparation of *Exo* 5

A stirred solution of *N*-ethylmaleimide (50 mmol) and furan (500 mmol) in dry benzene was heated at reflux for 18 hours. Benzene and excess furan were evaporated under vacuum at 60°C. The solid product was recrystallized in 91% yield from diethyl ether and dried under vacuum at room temperature. The product was determined to be pure *exo* isomer by ^{1}H NMR spectroscopy. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.50 (s, 2 H), 5.26 (s, 2 H), 3.51 (q, 2 H), 2.82 (s, 2 H), 1.15 (t, 3 H). High-resolution mass spectroscopy, electron ionization (HRMS, EI) calcd for $C_{10}H_{11}NO_3$ 193.074 g/mol, found 193.074 g/mol.

2.2.3 Preparation of *Endo* 5

A solution of *N*-ethylmaleimide (50 mmol) and furan (500 mmol) in dry benzene was allowed to react at room temperature for 4 days. Benzene and excess furan was evaporated under vacuum at 40°C. The solid product was washed with cold diethyl ether and dried under vacuum at room temperature to give a 89% yield. The product was determined to be pure *endo* isomer by ^{1}H NMR spectroscopy. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.39 (s, 2 H), 5.31 (d, 2 H), 3.49 (d, 2 H), 3.36 (q, 2 H), 1.03 (t, 3 H). HRMS (EI) calcd for $C_{10}H_{11}NO_3$ 193.074 g/mol, found 193.074 g/mol.

2.2.4 Polymer Characterization

^{1}H (300 MHz), ^{13}C (75 MHz) and ^{1}H-^{1}H COSY NMR spectra were obtained at with a Bruker DPX-300 NMR spectrometer. Gel permeation chromatography (GPC)

was performed with a Polymer Lab LC1120 high performance liquid chromatography (HPLC) pump equipped with a Waters differential refractometer detector. The mobile phase was tetrahydrofuran with a flow rate of 1 mL/min. Separations were performed with 10^5, 10^4, and 10^3 Å Polymer Lab columns. Molecular weights were calibrated versus narrow molecular weight polystyrene standards.

2.2.5 General Copolymerization Procedures

Catalyst **2** or **3** (4 μmol) was dissolved in 1 mL of CH_2Cl_2 and added to a solution of an equimolar mixture of a polar and non-polar monomer (1 mmol total) in 1 mL CH_2Cl_2. The reaction mixture was stirred for 12 hours at room temperature. The reaction was stopped with injection of 5 ml of CH_2Cl_2 containing a trace amount of ethyl vinyl ether. The polymer was precipitated in 30 ml of methanol except for the anhydride-functionalized polymers, which were precipitated in pentane. The polymers were recovered by filtration and dried overnight under vacuum at room temperature. The isolated yields were between 80-97% depending on starting monomer combinations.

Reactivity ratio values were obtained according to the following procedure. Five monomer mixtures with 1/9, 3/7, 5/5, 7/3, 9/1 cyclooctene to *endo* **5** ratios were prepared (1 mmol total) and dissolved in 2 mL CH_2Cl_2. Catalyst **2** (4 µmol) was added to each of these solutions. The polymerizations were stopped at low conversion by precipitation in excess methanol. The polymers were separated from methanol by centrifugation and dried overnight under vacuum at room temperature. The polymer composition values were obtained by ^{1}H NMR. Reactivity ratio values were obtained by nonlinear regression.[3]

2.2.6 Homopolymerization of *Endo* 5

Poly(*endo* **5**), which was used for the ^{1}H NMR analysis for structural comparisons of the corresponding homopolymers and copolymers, was prepared as follows. Catalyst **3** (10 μmol) was dissolved in 0.5 mL of CH_2Cl_2 and added to a solution of *endo* **5** (1 mmol) in 0.5 mL CH_2Cl_2. The reaction mixture was stirred for 12 hours at 35°C in a sealed reactor. The reaction was stopped with injection of 1 ml of CH_2Cl_2 containing a trace amount of ethyl vinyl ether. The polymer was precipitated in 10 ml of pentane. The polymer was recovered by centrifugation and removal of supernatant, followed by drying overnight under vacuum at room temperature. The isolated yield was 40%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.10 (s, trans), 5.82 (s, cis) (cis/trans = 33/67), 5.05 (br, cis), 4.52 (s, trans), (2H, cis/trans = 35/65), 3.52 (s, 2H), 3.30 (s, 2H), 1.14 (t, 3H).

2.2.7 Homopolymerization of Cyclooctene

Polycyclooctene, which was used for the ^{1}H NMR analysis for structural comparisons of the corresponding homopolymers and copolymers, was prepared as follows. Catalyst **2** (4 μmol) was dissolved in 1 mL of CH_2Cl_2 and added to a solution of cyclooctene (1 mmol) in 1 mL CH_2Cl_2. The reaction mixture was stirred for 2 hours at room temperature. The reaction was stopped with injection of 1 ml of CH_2Cl_2 containing a trace amount of ethyl vinyl ether. The polymer was precipitated in 20 ml of pentane. The polymer was recovered by filtration and dried overnight under vacuum at room temperature. The isolated yield was 85%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 5.36 (m, 2H), 2.02 (s, 4H), 1.34 (s, 8H).

2.2.8 Polymerization Monitoring by ^{1}H NMR and Rate Comparison Experiments

The sample solutions were prepared with 0.2 mmol of total monomer in 0.7 mL $CDCl_3$ in an NMR tube. For copolymerizations equimolar mixtures of monomers were used. Catalyst **2** or **3** (0.8 μmol) was dissolved in 0.1 mL of $CDCl_3$ and added to the monomer solution at room temperature. Rate comparison experiments were conducted by ^{1}H NMR. Data was collected every 2 minutes using naphthalene as an internal standard. It was not possible to probe the homopolymerization of *exo* **7** with the above monomer and catalyst concentrations due to polymer precipitation. Consequently, for these experiments a 1:10 catalyst to monomer ratio was used and the rate constant data was adjusted accordingly.

The preparation of ruthenium-cyclooctene chain-end species was performed by addition of cylooctene (60 μmol) to catalyst **2** (12 μmol) in 0.8 mL $CDCl_3$ and then allowed to react for 20 minutes at room temperature. The reaction of *endo* **5** with the resulting chain-ends was performed by adding an excess of *endo* **5** (0.2 mmol) to this solution. The preparation of ruthenium-*endo* **5** chain-end species was performed by addition of *endo* **5** (36 μmol) to catalyst **2** (12 μmol) in 0.8 mL $CDCl_3$ and then allowed to react for 20 minutes at room temperature. The reaction of cyclooctene with these chain-ends was performed by adding an excess cyclooctene (0.4 mmol) to this solution.

2.3 Results and Discussion

2.3.1 Determination of Alternating Microstructure

The ^{1}H and ^{13}C NMR spectra of the polymer resulting from the copolymerization of an equimolar mixture of *endo* **5** and cyclooctene using catalyst **2** indicated the absence of resonances for either homopolymer. This is most clearly seen by analysis of the olefinic region in the ^{1}H NMR spectrum that reveals resonances from

a mixture of *cis* and *trans* isomers of an asymmetric carbon-carbon double bond of a regular alternating structure (Figure 2.1).

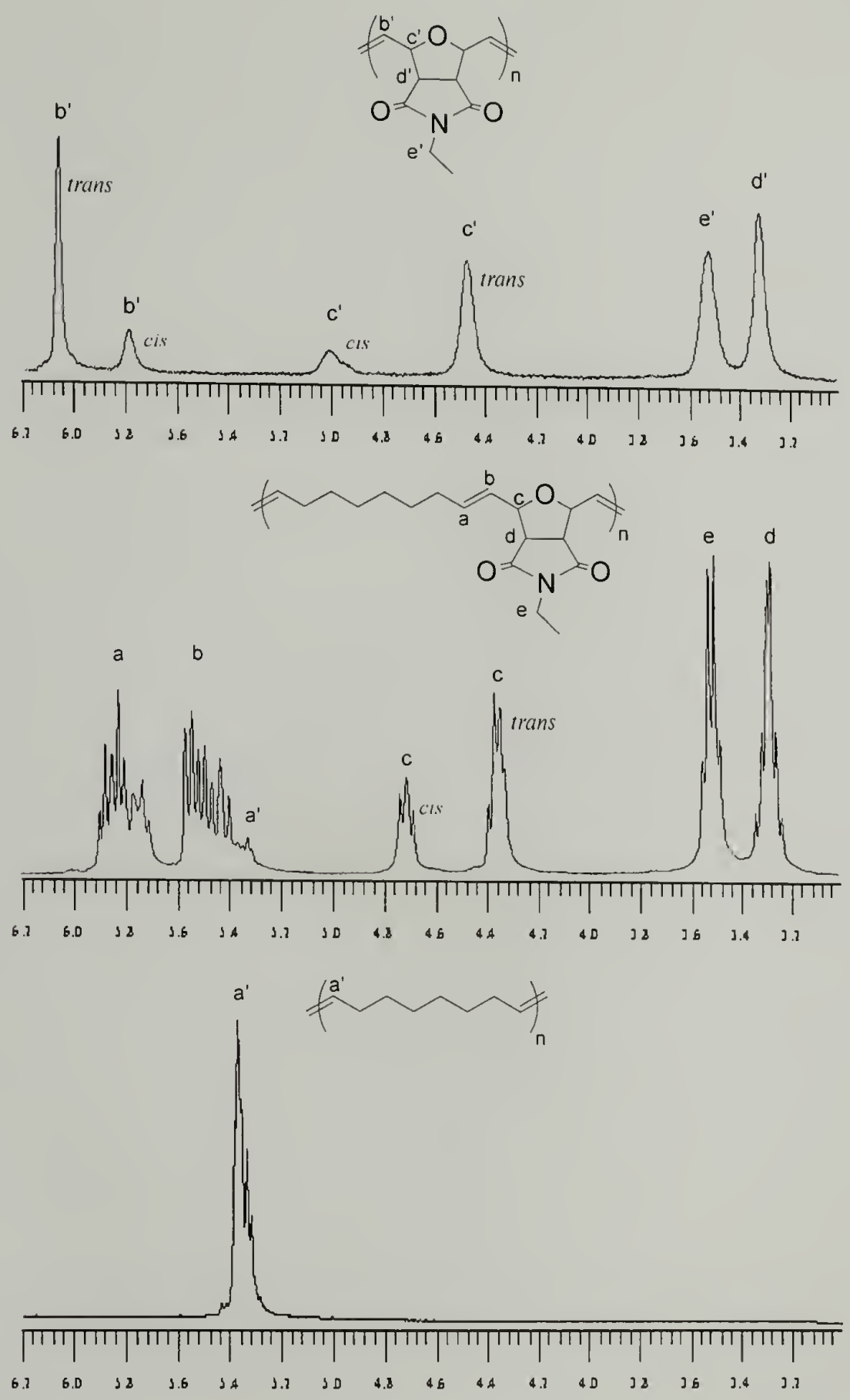


Figure 2.1 ^{1}H NMR spectra of the homopolymer of *endo* **5** (Top), alternating copolymer of *endo* **5** and cyclooctene (Middle) and the homopolymer of cyclooctene (Bottom) in $CDCl_3$.

Changing the reaction time, catalyst or total monomer concentrations did not affect the resulting high levels (>98%) of alternation in the copolymer. Molecular weights were tunable from 10,000 to approximately 200,000 g/mol depending on the ratio of catalyst to monomers, from 1/200 to 1/1500 respectively, with polydispersity values near 2. Inspection of the ^{1}H-^{1}H COSY NMR spectrum clearly shows the internal connectivity of a repeat unit that results from an alternating polymerization of *endo* **5** and cyclooctene (Figure 2.2).

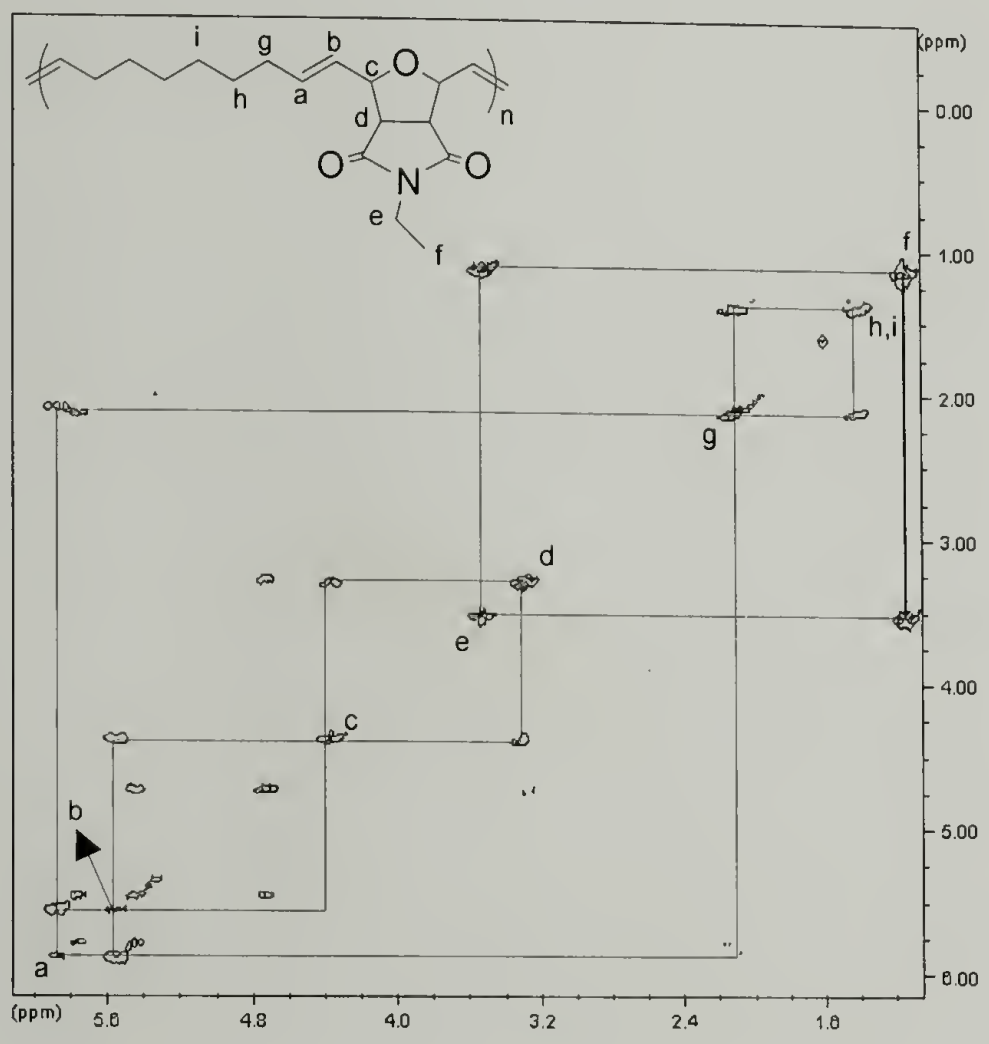


Figure 2.2 ^{1}H-^{1}H COSY NMR spectrum of alternating copolymer of *endo* **5** and cyclooctene. The rectangles show the off-axis peaks establishing the connectivity. Dashed lines represent the cis isomer.

2.3.2 Alternating Copolymerization of *Endo* 5 and Cyclooctene

To quantify the tendency towards alternation, the reactivity ratios for the copolymerization of *endo* **5** and cyclooctene were calculated using copolymer

composition equation.[3] As expected the reactivity ratios are very small, and the corresponding reactivity ratio product approaches zero (Table 2.1). In an ideal alternating copolymerization these values become zero representing the absence of any homopolymerization.

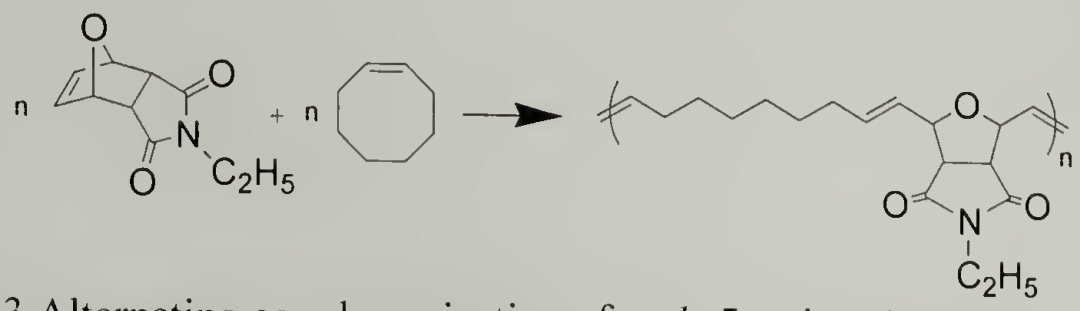


Figure 2.3 Alternating copolymerization of *endo* **5** and cyclooctene.

Table 2.1 Reactivity ratios for the copolymerization of cyclooctene and *endo* **5** using catalyst **2** and the corresponding reactivity ratio product.

$r_{cyclooctene}$	0.08 $^{+}/_{-}$ 0.02
$r_{endo\ 5}$	0.04 $^{+}/_{-}$ 0.02
Reactivity ratio product	$0.001 < r_{cyclooctene}\ r_{endo\ 5} < 0.006$

The *in situ* monitoring of the copolymerization was performed in a series of NMR tube experiments. The rate of disappearance of each monomer was observed to be equal. Furthermore, it was also observed that only an alternating structure appeared from the very onset of polymerization. For comparison the homopolymerizations of *endo* **5** and cyclooctene were also monitored by ^{1}H NMR. The copolymerization of *endo* **5** with cyclooctene was observed to be faster than homopolymerization of *endo* **5** but slower than homopolymerization cyclooctene (Figure 2.4). When (ln[monomer] – ln[monomer]$_0$) data was plotted versus time, linear functions were obtained for the copolymerization and either of the homopolymerizations. From these calculations the rate constants were found to be 2.3×10^{-3} sec^{-1} for cyclooctene homopolymerization,

4×10^{-5} sec^{-1} for *endo* **5** homopolymerization and 4×10^{-4} sec^{-1} for their copolymerization. Although only an alternating structure is observed from an equimolar monomer mixture, the rate of copolymerization is slower than cyclooctene homopolymerization.

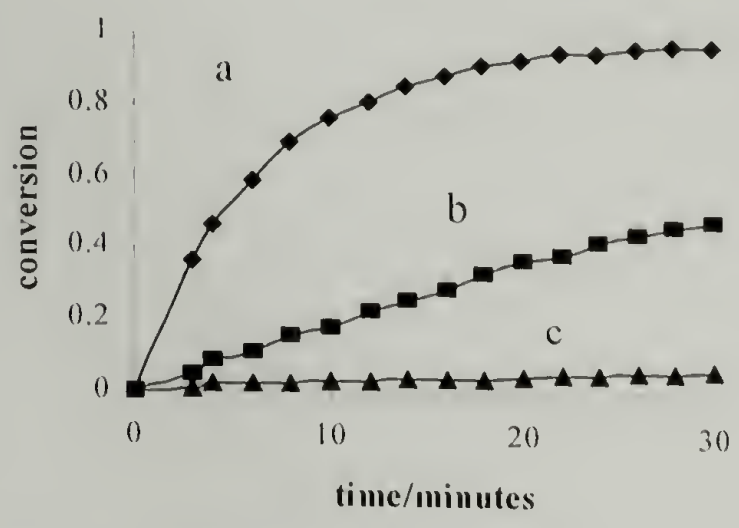
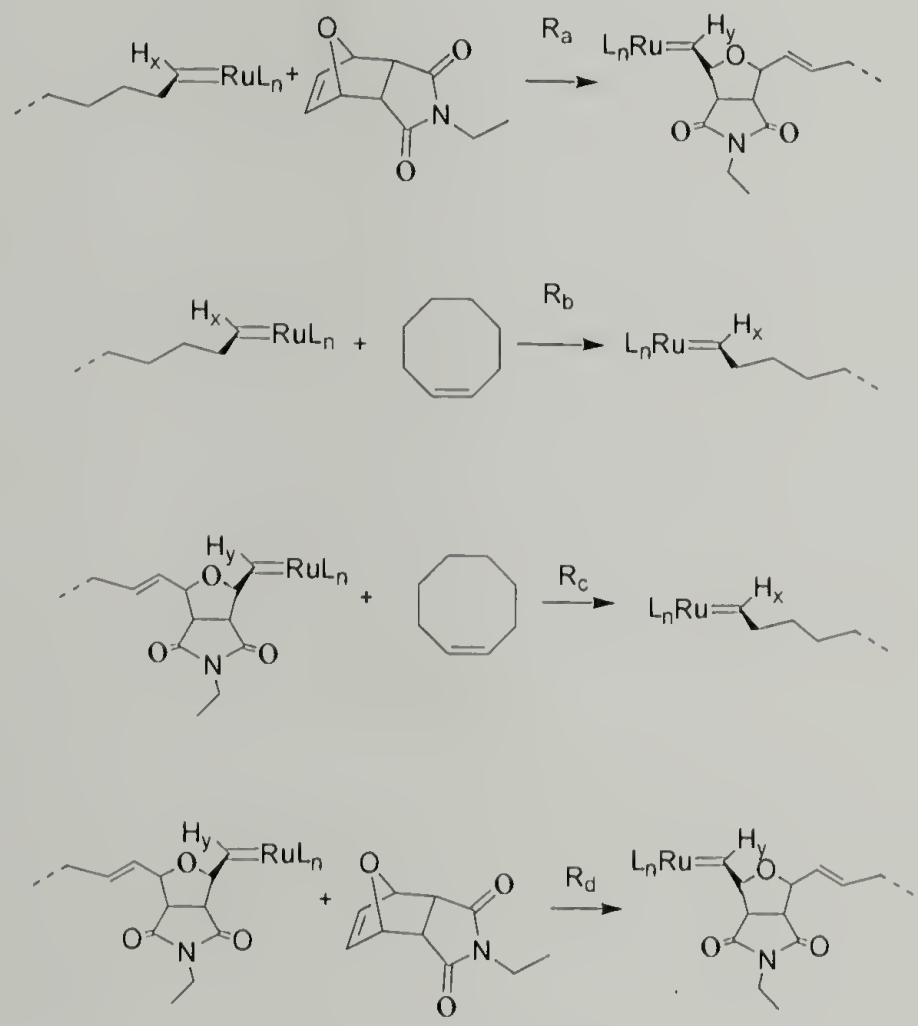


Figure 2.4 The comparison of the rates of cyclooctene homopolymerization (a), copolymerization of an equimolar mixture of cyclooctene and *endo* **5** (b) and homopolymerization of *endo* **5** (c) using catalyst **2**.

An alternating copolymerization includes two different propagation reactions. In this particular case, one step is the addition of *endo* **5** to a ruthenium-cyclooctene chain end and the other is the addition of cyclooctene to a ruthenium-*endo* **5** chain-end. To resolve these two propagation rates both propagating species were independently generated and then allowed to react with the other monomer. Addition of excess cyclooctene to catalyst **2** in $CDCl_3$ consumed all cyclooctene and initial catalyst in less than 20 minutes generating ruthenium carbene species at the chain-ends of cyclooctene oligomers as observed by ^1H NMR. An excess of *endo* **5** was added to this solution. The reaction rate was observed from the disappearance of the resonance for the ruthenium carbene proton of the ruthenium-cyclooctene chain-end (19.3 ppm) and appearance of a resonance for the ruthenium-*endo* **5** chain-end (18.6 ppm). In a similar fashion the ruthenium-*endo* **5** chain-ends were generated in an NMR tube, an excess of cyclooctene

was added to this solution. The comparison of the rates for the different propagating steps are presented in Figure 2.5 The observation that R_a is approximately two times faster then R_b would result in a preference for an alternating structure. On the other hand the observation that R_c is more than ten times slower than R_b explains why the overall rate for copolymerization of cyclooctene and *endo* **5** is slower than cyclooctene homopolymerization.



$R_a > R_b > R_c > R_d$

Figure 2.5 Comparison of the rates for *endo* **5** addition to a ruthenium-cyclooctene chain-end (R_a), cyclooctene addition to a ruthenium-cyclooctene chain-end (R_b), cyclooctene addition to a ruthenium-*endo* **5** chain-end (R_c) and *endo* **5** addition to a ruthenium-*endo* **5** chain-end (R_d). Both chain-ends were derived from catalyst **2**.

2.3.3 Alternating Copolymerization of *Exo* 7 and Cyclooctene

The conversion versus time data for the copolymerization of *exo* **7** with cyclooctene and their homopolymerizations were obtained in a similar manner. The comparison of the plots revealed that the copolymerization of *exo* **7** with cyclooctene is

faster than homopolymerization of either monomer (Figure 2.6). This result is consistent with the resulting alternating distribution. The rate constant was 5×10^{-4} sec^{-1} for *exo* **7** homopolymerization, 2.3×10^{-3} sec^{-1} for cyclooctene homopolymerization and 2.2×10^{-2} sec^{-1} for their copolymerization. The value for the copolymerization rate constant is presumably a lower limit as the first data point the polymerization was at very high conversion. Unlike the homopolymer of *exo* **7** this alternating copolymer is soluble in common organic solvents. An importance of this alternating copolymer is the precisely separated anhydride functionalities, which provide the opportunity for further functionalization.

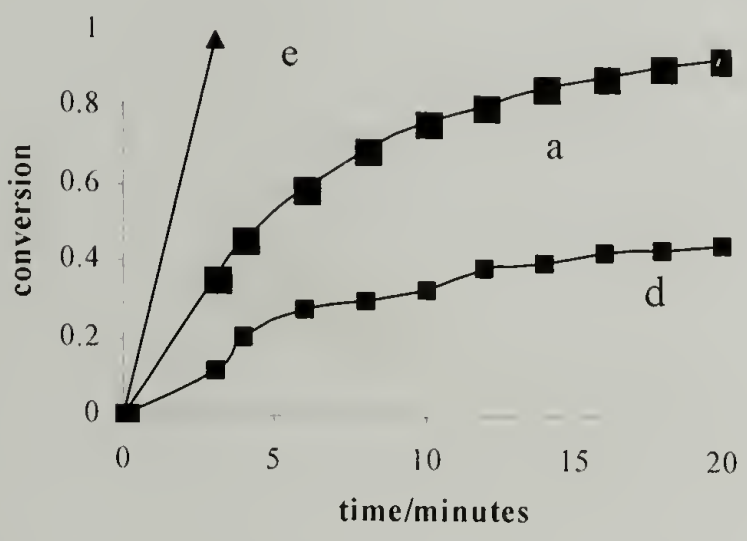


Figure 2.6 The comparison of the rates of homopolymerization of cyclooctene (a), *exo* **7** (d) and their copolymerization from an equimolar mixture (e) using catalyst **2**.

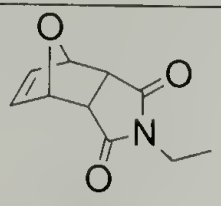
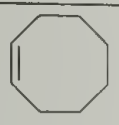
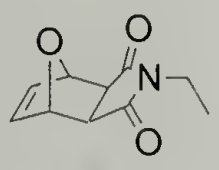
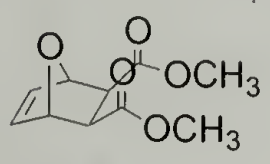
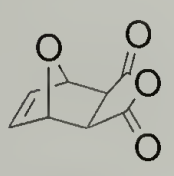
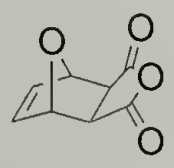
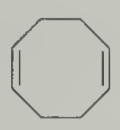
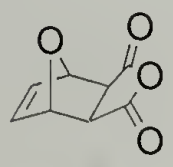
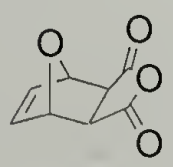
2.3.4 Generality of Alternating Copolymerization

2.3.4.1 Monomer Structure

Oxanorbornenes are known to be more reactive than cyclooctene in ring-opening metathesis homopolymerization due to their higher ring strain. Rather than obtaining a block copolymer structure that would have resulted from preferential consumption of one monomer prior to consumption of the other, we have observed alternating structures for the copolymerization of cyclooctene with either *endo* **5**, *exo* **6**

or *exo* **7** (Table 2.2). The change from the *endo* to *exo* isomer of **5** decreases the tendency towards alternation. This result can be understood if the approach of a propagating metal center to an oxanorbornene derivative to form a metallocyclobutane intermediate is accepted to be from the *endo* face of the carbon-carbon double bond. Thus the more hindered *endo* isomer of **5** undergoes a slower homopropagation relative to the cross-propagation with cyclooctene. After cyclooctene has ring-opened, the chain- end becomes less sterically hindered and preferentially propagates by the addition of the higher ring strain *endo* **5**. In comparison, *exo* **5** has a less hindered carbon-carbon double bond and consequently undergoes faster homopropagation in the presence of cyclooctene leading to a less precisely alternating structure. The copolymers prepared from cyclooctene and *endo* **5** or *exo* **7** are observed to be the closest to perfectly alternating copolymers. In their ^{1}H NMR spectra, a small peak arising from a homopolymer of only one of the comonomers (e.g. a' in Figure 2.1, Middle) indicates a possible stoichiometric mismatch in the reaction feed rather than tendency to random monomer addition in which case the presence of both types of homopolymers would be observed. In the case of *exo* **6** the alternating diad content was slightly decreased to be 91%. One difference of this monomer is the mobility of the 2,3-substituents that could bring an additional steric hinderence when compared to the rigid five-membered ring substitutions at the 2 and 3 positions of *endo* **5**, *exo* **5**, and *exo* **7**. A balanced effect of increased steric hinderence and decreased polar character of dimethyl esters compared to the anhydride functionality of *exo* **7** could explain the above result for attempted alternating copolymerization of cyclooctene and *exo* **6**.

Table 2.2 Percentage of alternating diads[a] resulting from the copolymerizations of different monomer combinations for catalysts **2** and **3**.

Monomers[b]		**2**	**3**
 *endo* **5**		98	85
 *exo* **5**		80	70
 *exo* **6**		91[d]	60[d]
 *exo* **7**		96	75[d]
 *exo* **7**		92[d]	[c]
 *exo* **7**		85	70[d]
 *exo* **7**		40	[c]

[a] Based on ^{1}H NMR spectra. [b] Equimolar mixtures of monomers. [c] Not determined. [d] %5 error margin due to poor resolution of the peaks.

When cyclooctene is replaced by cyclooctadiene, cyclopentene or norbornene the alternating copolymer structure begins to have more irregularities indicating the effect of different ring strains. Cyclooctadiene has the same ring size as cyclooctene providing a steric hindercnce very similar to cyclooctenc. However the second carbon-carbon unsaturation on cyclooctadiene ring is expected to alter the ring strain and also act as an additional coordination site for the approaching ruthenium catalyst. With these structural features cyclooctadienc is known to be a slower ROMP monomer compared to cyclooctenc. All these factors would bc expected to contribute in the decreased alternation character of cyclooctadiene-*exo* 7 copolymer. In the case of cyclopentene the changes in ring strain and steric hindcrence, related to thc dccreased ring size, are the expected reasons for further dccreascd alternation bchavior in its copolymerization with *exo* 7. In the copolymcrization of norbornene and *exo* 7 the tendency towards alternation is lost as thc norbornene has a very similar ring strain to oxanorbornene while lacking the 2,3-disubstitution. Overall, these results indicate that in the ruthenium catalyzed ring-opening mctathesis copolymerization, a balancc of ring strain and steric hinderencc of the comonomers are crucial factors for achieving alternation.

2.3.4.2 Comonomer Feed Ratio

In sections 2.3.2 and 2.3.3, the tendency toward alternation has been shown to be related to corresponding relative propagation rates. It is also well known that the polymerization rate of a monomer, in homo- or copolymerizations, is directly related to its concentration in the polymerization solution. In order to further test the extent of the tendency toward alternation an uneven comonomer feed ratio was used in the copolymerization of *exo* 7 and cyclooctene. This comonomer combination has been shown to copolymerize in a highly alternating fashion in the case of equal feed ratios

(Table 2.2). In order to eliminate any possible early precipitation that can be caused by high *exo* **7** content in the copolymer this comonomer was used as the minor component. When **2** catalyzed copolymerization of *exo* **7** and cyclooctene, with a comonomer ratio of 1 to 9 respectively, was monitored *in situ* using ^{1}H NMR spectroscopy it was observed that first an alternating copolymer structure appears. Peaks from cyclooctene homopolymer sequences do not appear until *exo* **7** is consumed. This experiment shows that the rate of alternating propagation is much higher than cyclooctene homopropagation so that it can not be suppressed even at 10% *exo* **7** comonomer feed ratio. This result is in good agreement with the very high copolymerization rate shown in Figure 2.4. If chain transfer through cross-metathesis reactions can be suppressed, for example by short polymerization times, then the resulting copolymer would likely be a blocky copolymer, where one block is a poly(*exo* **7**-*alt*-cyclooctene) copolymer, and the other is polycyclooctene homopolymer (Figure 2.7).

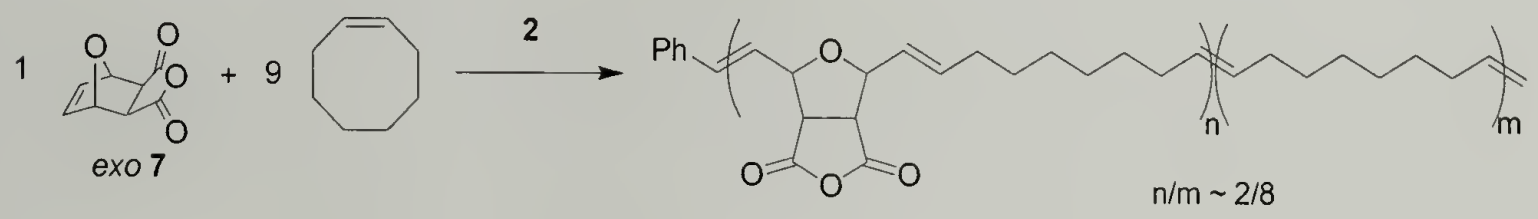


Figure 2.7 Likely blocky copolymer structure resulting from the copolymerization of *exo* **7** and cyclooctene with a feed ratio of 1 to 9 respectively.

2.3.4.3 Catalyst

To probe the generality of alternating copolymerization with different catalysts copolymerization of the monomers listed in Table 2.1 were performed using **3**. A decrease in the tendency towards alternation was observed in all cases (Table 2.1). For example, the ^{1}H NMR of the copolymer obtained from the copolymerization of an equimolar mixture of *endo* **5** and cyclooctene is shown in Figure 2.8. The resonances

labeled as a', b' and c' show the presenee of symmetric unsaturations resulted from homopropagation, a, b and c are asymmetric units which result from cross-propagation.

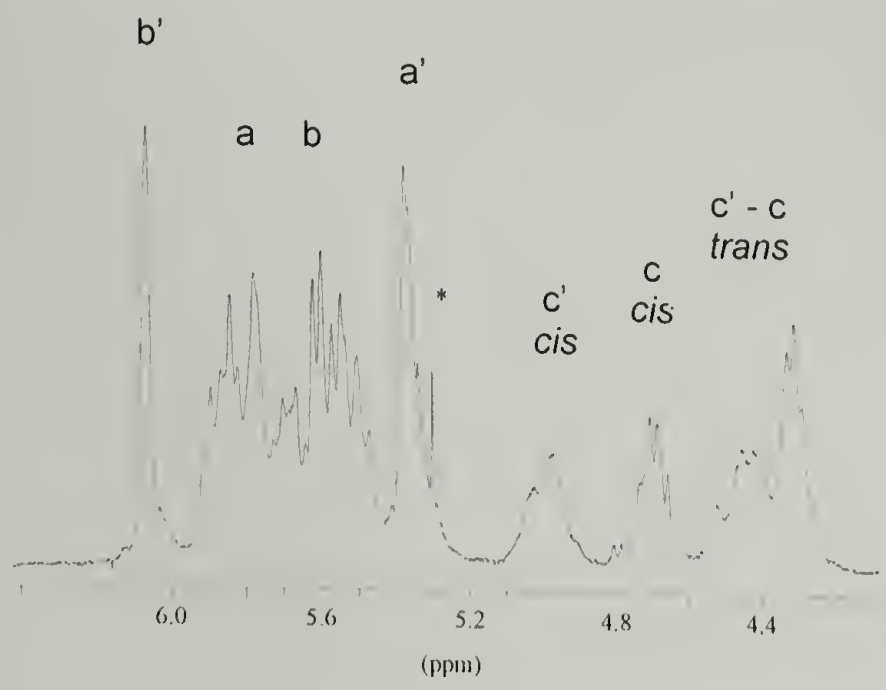


Figure 2.8 ^{1}H NMR speetrum of the eopolymer of *endo* **5** and eyelooctene made from an equimolar mixture using eatalyst **3**. The peak assignments are the same as in Figure 2.1 and 2.2. (* = CH_2Cl_2).

This ean be explained by the known higher activity and greater steric tolerance of this eatalyst, whieh results in less seleetivity during copolymerization.[10] A very similar result was obtained from the copolymerization of an equimolar mixture of *exo* **7** and eyelooetene using **4**, the highly aetive bromo-pyridine substituted Grubbs-Love catalyst. The pereentage of the alternating diads was about 85%. One advantage of **4** is very fast initiation rates and lack of ehain transfer in the polymerization of substituted norbornenes.[11] The eopolymerization that was performed at room temperature resulted in relatively large moleeular weight distributions, with PDI values 2 to 3. The relatively less hindered earbon-earbon double bond of the alternating diads would likely allow ehain transfer through eross-metathesis to baekbone unsaturations. However when the eopolymerizations were run at -30°C PDI values below 1.2 were obtained revealing the ability of minimizing ehain transfer reaetions by lowering the reaction temperature.[11]

This result is significant since it is generally challenging in polymer chemistry to prepare alternating copolymers with a high level of control over molecular weight distributions. Overall copolymerizations performed using catalyst **3** and **4** did not result in perfectly alternating copolymers. However it is worth noting that the resulting copolymers were not random. There were certain degrees of tendency towards alternation, resulting in about 85% alternating diads, rather than 50% in a perfectly random copolymerization. Here it can be predicted that more appropriate comonomer combinations, for example involving more hindered norbornene derivatives, could possibly result in less defective alternating copolymers from polymerizations catalyzed by **3** or **4**.

The attempted copolymerization of *exo* **6** and cyclooctene using **1**[12] resulted in a copolymer structure with ^{1}H NMR resonances arising predominantly from homopolymer sequences. Although a significant amount of asymmetric unsaturations that result from cross-propagation was also observed. The resulting polymer is most likely a tapered-block copolymer.

2.4 Conclusion: Synthetic Utility of Alternating ROMP

In summary, the alternating copolymerization of 2,3-difunctionalized 7-oxanorbornene derivatives with nonpolar cyclic olefins via ring-opening metathesis has been demonstrated. This new method brings a number of significant advantages to ROMP-based polymer synthesis. First, alternating ROMP holds promise for preparing well-defined copolymers with tailorable polar functionalities regularly separated by nonpolar spacers, a unique polymer microstructure. Second, alternating ROMP facilitates, and in some cases allows for the polymerization of sterically encumbered monomers that do not undergo homopolymerization. A good example is *endo* 2,3-

difunctionalized norbornene derivatives, which do not homopolymerize or very slowly homopolymerize depending on the substituents. Another example is a literature report referring to our published work,[7] that describes the alternating copolymerization of cyclooctene with a norbornene derivative carrying a large substituent, a ruthenium hydrogenation catalyst (Figure 2.9).[13]

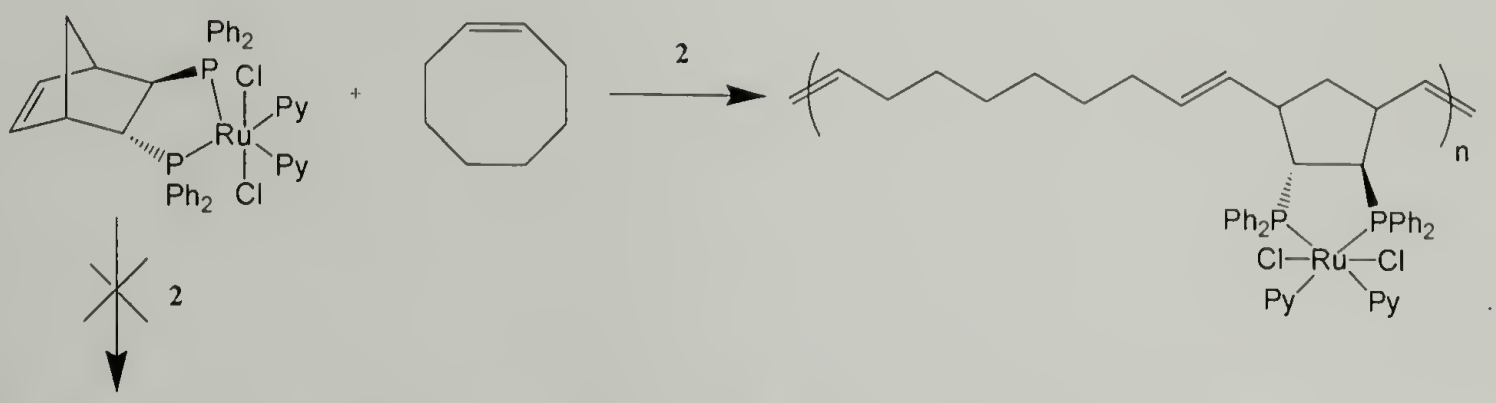


Figure 2.9 Preparation of polymeric hydrogenation catalyst through alternating ROMP of catalyst functionalized norbornene derivative and cyclooctene.[13]

Although very active and functional group tolerant catalyst systems are available, the steric limitations of monomers carrying novel functional groups will always be a consideration in monomer design and polymer preparation. This point is very significant as it reveals that the alternating ROMP approach goes hand-to-hand with catalyst systems that are compatible with a variety of functionality, in preparing polymers with large and/or complex functionalities. Furthermore, alternating ROMP is a good strategy to afford organic solvent soluble polymers with polar functionalities. Polymers carrying highly polar functionalities typically have limited solubilities in organic solvents. On the other hand many catalyst systems, including Grubbs catalyst, are very solvent selective and decompose in polar solvents. Hence the ROMP of polar monomers commonly results in early precipitations of low molecular weight products. Alternating ROMP of these type of monomers with nonpolar cyclic olefins (e.g.

cyclooctene) provide excellent solubility to the resulting high molecular weight polymers in common ROMP solvents such as dichloromethane, chloroform, and toluene. We therefore envisage using ring-opening metathesis copolymerization as a general convenient strategy for introducing varying levels of polar functionalities into polyalkenamers.

2.5 References

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CHAPTER 3

MODULAR NORBORNENE DERIVATIVES FOR THE PREPARATION OF WELL-DEFINED AMPHIPHILIC POLYMERS

3.1 Introduction

Polymers are commonly classified as hydrophilic or hydrophobic when referring to their overall physiochemieal properties. Amphiphilic polymers, on the other hand, carry a balaneed combination of polar, hydrophilic, and nonpolar, hydrophobic, charaeters and exhibit strong interfaeial interactions. Amphiphilie eharaeter is an important maeromoleeular asset when polymers are designed to interaet at interfaces of polar and nonpolar media. Synthetic amphiphilic polymers are generally prepared through bloek, random, or alternating eopolymerizations of polar and nonpolar monomers. In the ease of amphiphilie bloek eopolymers, the amphiphilicity is at the macromoleeular level resulting in unique properties such as solvophobieally driven mieelle formations.[1-3] Random or alternating eopolymers exhibit amphiphilic properties along their baekbone where hydrophobie and hydrophilie groups are in elose proximity at the molecular level.[4,5] However, for these types of polymerizations, the choiee of eomonomers and the level of control over the molecular weights bring limitations when eompared to homopolymerizations. Homopolymerizations eould provide diversity of the hydrophobie and hydrophilie eharaeter if both attributes are present in the repeating unit. The initial focus of this ehapter is the preparation and homopolymerization of a novel elass of eyelie olefin monomers with amphiphilie eharaeter where the amphiphilieity of the resulting polymer is tuned at the repeating unit level, giving rise to a polymer baekbone strueture with regularly spaeed hydrophilie and hydrophobie groups. The moleeular weight of the amphiphilie polymer is independently eontrolled

through the choice of polymerization procedure. Further studies involving the random and block copolymerizations of this class of monomers will also be presented in order to extend the synthetic capabilities towards increased structural control, and broaden the scope of possible applications.

In this study the starting point for monomer design is based on widely used norbornene derivatives. Norbornene derivatives having 2-mono or 2,3-difunctionalization are known to be excellent monomers for ROMP. They have been used in the preparation of a wide range of polymeric structures.[6] Because of the strained nature of the norbornene ring these are active monomers for living ROMP resulting in narrow polydispersity polymers in addition to tolerating the presence of large side groups. Using various norbornene derivatives, polymers bearing a variety of side groups have been prepared via ROMP. Examples include polynorbornene derivatives carrying oligopeptides,[7] oligonucleotides,[8] anti-cancer drugs,[9] saccharides,[10] dendrons,[11] and polymeric side groups.[12,13] Functionalized norbornene derivatives are readily prepared via Diels-Alder cycloaddition of a diene, most generally furan or cyclopentadiene, to a dienophile possessing a desired functional group.[6] This procedure affords an *endo* or *exo* 2- or 2,3-functionalized norbornene derivative (Figure 3.1). *Endo* isomers are known to be poor monomers for ROMP, presumably because of the increased steric crowd around the polymerization active carbon-carbon double bond.

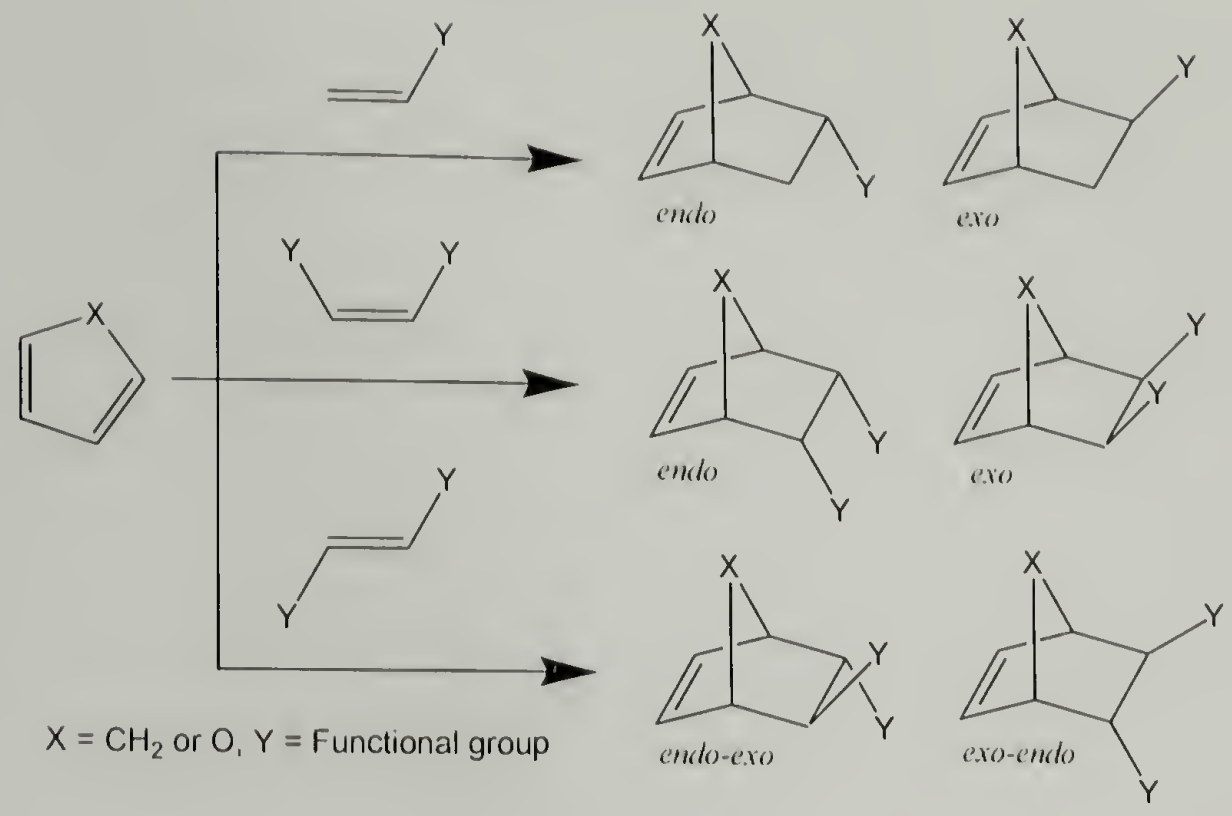


Figure 3.1 General schemes for the cycloaddition reactions of furan, or dicyclopentadiene, with 1-, or 1,2-functionalized olefins producing *exo* or *endo* forms of 1-, or 1,2-functionalized norbornene derivatives.

In this study, the task of preparing a monomer structure with dual functionality, in this case a hydrophilic and a hydrophobic group, lead us to investigate the preparation and polymerization of modular norbornene derivatives with an additional functionality on the 7 position of the ring (Figure 3.2). Using this general strategy, two complementary functionalities can be introduced into the monomer structure and the properties of the resulting amphiphilic polymer can thus be fine-tuned.

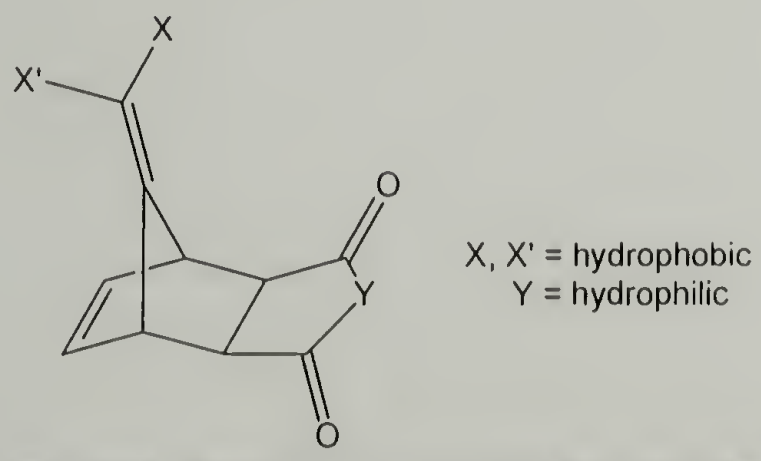


Figure 3.2 General structure of amphiphilic modular norbornene derivatives.

Advances in catalyst development made a series of metathesis catalyst available through commercial sources or facile preparations. The molybdenum based Schrock catalyst **1**, a very active catalyst, allows for living polymerizations (Figure 3.3).[14-16] More recently Grubbs catalyst derivatives, **2**, **3**, and **4**, with improved stabilities, also show high activities.[17,18] The latest catalyst, **4**, bearing labile 3-bromo pyridine ligands, exhibits very high activities in addition to fast initiation rates that allow for the preparation of narrow polydispersity polymers.[18,19] Therefore, with the availability of powerful metathesis catalysts and the suitable choice of monomer, amphiphilic polymers with controlled molecular weights and narrow molecular weight distributions can be prepared. This chapter focuses on the synthesis and ROMP of modular norbornene derivatives to obtain novel well-defined amphiphilic polymers.

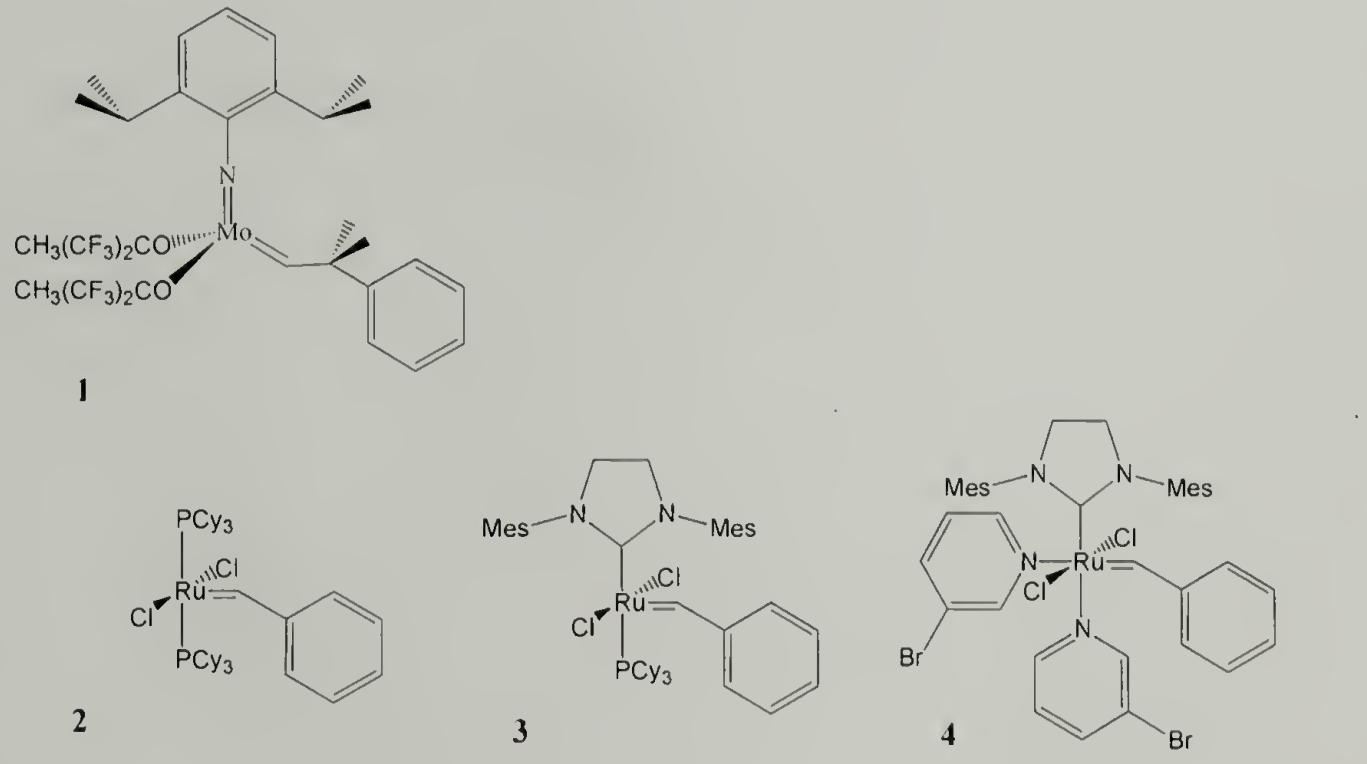


Figure 3.3 Catalysts **1**, **2**, **3** and **4**.

3.2 Experimental Section

3.2.1 Materials

2,6-diisopropylphenylimidoneophylidenemolybdenum (VI) bis(hexafluoro-*tert*-butoxide) (**1**) ,[14-16] $RuCl_2(=CHPh)(PCy_3)_2$ (**2**),[20] and (tricyclohexylphosphine) (1,3-dimesitylimidazolidine-2-ylidine)benzylideneruthenium dichloride (**3**)[21] were purchased from Strem Chemical. Cyclopentadiene for the synthesis of fulvene derivatives was obtained by the thermally induced cracking of dicyclopentadiene at 150°C, followed by distillation. Fulvene derivatives,[22] compound **8**,[23] compound **14**,[24] and $[(H_2Imes)(3\text{-Br-py})_2\text{-}(Cl)_2Ru{=}CHPh]$ (**4**)[18] were prepared according to literature procedures. All other reagents were obtained from Aldrich. Deuterated chloroform, dichloromethane and toluene were passed through columns of basic activated alumina prior to use.

3.2.2 Instrumentation

^1H (300 MHz), and ^{13}C NMR (75 MHz) spectra were obtained on a Bruker DPX-300 NMR spectrometer. Gel permeation chromatography (GPC) was performed with a Polymer Lab LC1120 high-performance liquid chromatography (HPLC) pump equipped with a Waters differential refractometer detector. The mobile phase was tetrahydrofuran (THF) or dimethylformamide (DMF) with a flow rate of 1.0 mL/min and 0.5 mL/min respectively. Separations were performed with 10^5, 10^4, and 10^3 Å Polymer Lab columns. Molecular weights were calibrated versus narrow molecular weight polystyrene standards. Aqueous GPC was performed using a Kratos Spectroflow 400 Pump, Shimadzu RID-6A RI detector and TSK-GEL column set (2x GMPWXL, 1x G3000PWXL, and 1x G2000SW). Phosphate buffer (0.035 M, pH = 8.2, I = 0.4) was used as an eluent at a flow rate of 1.0 mL/min. The system was calibrated with narrow poly(ethylene oxide) standards.

3.2.3 Preparation of 9

A literature procedure for the cobalt catalyzed maleic anhydride-maleimide transformation was adapted for the synthesis of monomer **9**.[25] Mono protected diamine **14** (1.57 g, 9.8 mmol) was added to **8** (1 g, 4.9 mmol) in DMAc (N,N-Dimethylacetamide, 6 mL) at 60°C and stirred for 20 minutes. A catalytic amount of cobalt acetate (0.1 mmol) dissolved in 4 mL DMAc, was added to this mixture followed by the addition of acetic anhydride (5 mmol). The reaction mixture was stirred for 4 hours at 80°C. After cooling to room temperature the solution was diluted with ethyl acetate, washed with water and dilute HCl (5 wt%), dried, and evaporated under reduced pressure to afford 92% yield of **9** with an 88:12 *exo-endo* ratio. Recrystallization from cold diethyl ether afforded pure *exo* isomer **9** (56%). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.42 (2H, t, J = 2.0 Hz), 4.78 (1H, s), 3.72 (2H, t, J = 1.9 Hz), 3.56 (2H, t, J = 5.6 Hz), 3.20 (2H, q, J = 5.3 Hz), 2.74 (2H, s), 1.53 (6H, s), 1.43 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 177.8, 155.8, 140.8, 137.8, 115.5, 79.6, 48.0, 45.7, 39.5, 38.4, 28.4, 19.7. Elemental analysis for $C_{19}H_{26}N_2O_4$ (346.43 g/mol) calculated: C, 65.92; H, 7.51; N, 8.09. Found: C, 65.73; H, 7.48; N, 7.95.

3.2.4 Preparation of 10

The Diels-Alder reaction between isopropylfulvene[22] (0.25 M) and maleic anhydride (0.25 M) was performed in ethyl acetate at 90°C for 12 hours in a sealed pressure tube. Upon removal of ethyl acetate under reduced pressure, the adduct (**10**) was obtained in high yield as an oil with an 80:20 *exo-endo* ratio. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.46 (2H, m), 4.82 (*exo*, 1H, d, J = 9.7 Hz), 4.64 (*endo*, 1H, d, J = 9.4 Hz), 3.94 (*endo*, 1H, s), 3.87 (*exo*, 1H, s), 3.60 (*endo*, 1H, s), 3.55 (*endo*, 2H, dd, J = 3.0

Hz, 4.2 Hz), 3.51 (*exo*, 1H, s), 3.05 (*exo*, 2H, dd, J = 8.1 MHz, 7.7 Hz), 2.30 (1H, m), 0.91 (6H, d, J = 5.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 171.4, 171.2, 171.1, 167.4, 149.5, 142.8, 137.7, 137.2, 135.9, 135.2, 121.5, 117.9, 49.0, 48.9, 48.5, 46.5, 46.3, 44.1, 28.2, 23.2, 22.9. Electron ionization (EI) high-resolution mass spectroscopy (HRMS) calcd for $C_{13}H_{14}O_3$ 218.0943 g/mol, found 218.0942 g/mol.

3.2.5 Preparation of 11

The same procedure that was used for the preparation of **9** from **8** was used for the preparation of **11** from **10** to afford 90% yield of **11** with an 85:15 *exo-endo* ratio. Recrystallization in cold diethyl ether afforded pure *exo* isomer of **11** (40%). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.42 (2H, m), 4.81 (1H, s), 4.68 (1H, d, J = 9.4 Hz), 3.72 (1H, s), 3.55 (2H, t, J = 5.6 Hz), 3.36 (1H, s), 3.29 (2H, broad), 2.76 (2H, dd, J = 10.2 Hz, 7.5 Hz), 2.24 (1H, m), 1.42 (9H, s), 0.88 (3H, d, J = 6.7 Hz), 0.79 (3H, d, J = 6.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 177.8, 156.0, 144.4, 138.0,137.3, 120.7, 79.5, 49.0, 47.9, 44.6, 39.2, 38.7, 28.5, 28.2, 23.6, 23.2. Repeated elemental analyses resulted in low carbon content. Elemental analysis for $C_{20}H_{28}N_2O_4$ (360.213 g/mol) calculated: C, 66.64; H, 7.83; N, 7.77. Found: C, 65.96, 65.66; H, 7.92, 7.74; N, 7.70, 7.68. HRMS (EI) calcd for $C_{20}H_{28}N_2O_4$ 361.213 g/mol, found 361.214 g/mol.

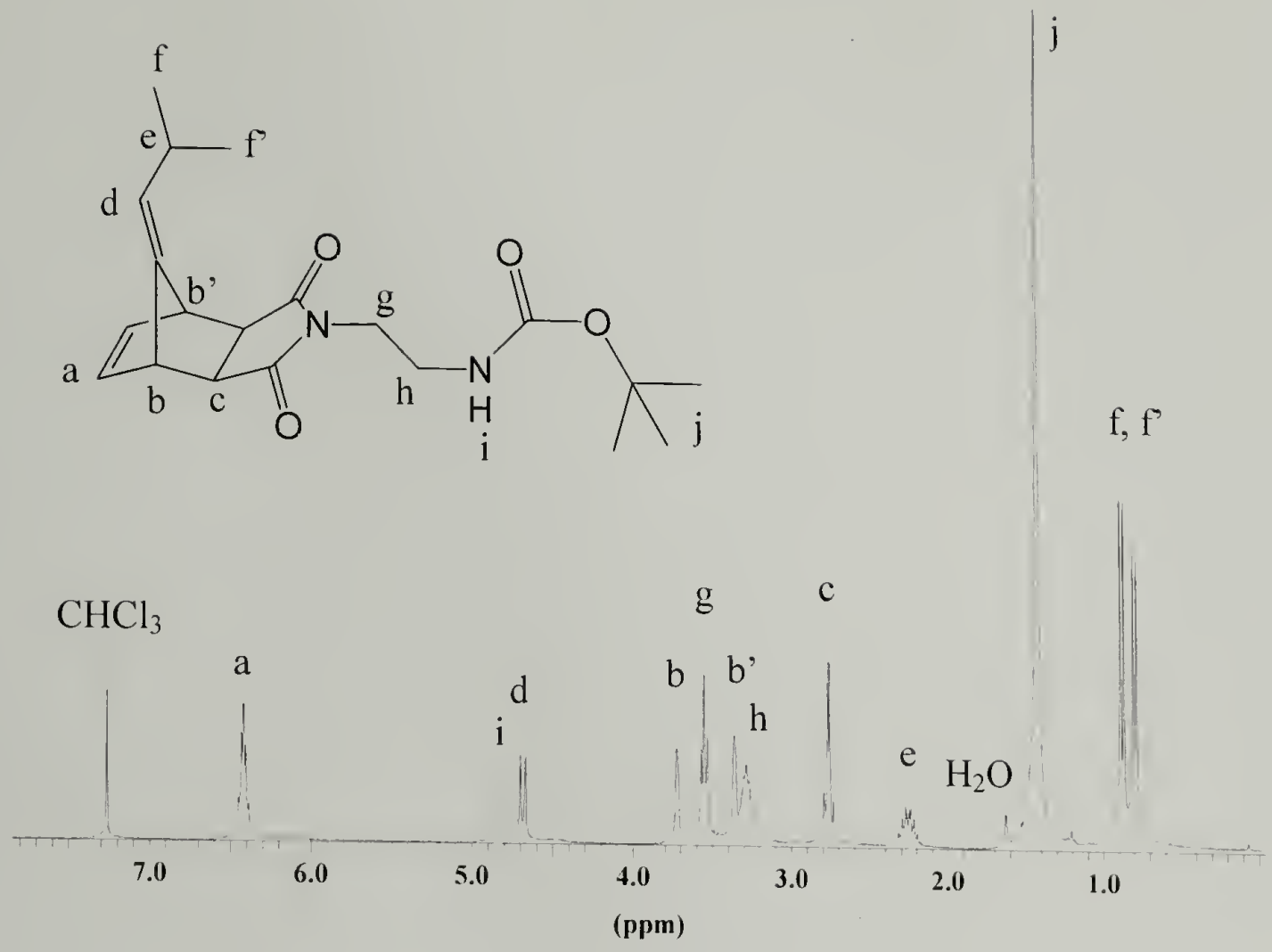


Figure 3.4 ^{1}H NMR spectrum of **11** in $CDCl_3$.

3.2.6 Preparation of 12

To a solution of 4-heptanone (20 mmol, 2.28 g) and cyclopentadiene (20 mmol, 1.32 g) in methanol (20 mL) was added pyrrolidine (20 mmol, 1.42 g). The mixture was stirred at room temperature for 1 hour and then acetic acid was added (20.1 mmol, 1.21 g) to quench the reaction. The reaction mixture was diluted with ether (50 mL) and water (50 mL). The ether portion was separated, washed with water (50 mL) and brine (50 mL), and dried over $MgSO_4$. Ether was removed under reduced pressure and the product, di-n-propylfulvene, was used without further purification for the cycloaddition with maleic anhydride. The Diels-Alder reaction between di-n-propylfulvene (20 mmol, 3.24 g) and maleic anhydride (20 mmol, 1.96 g) was performed in ethyl acetate (50 mL)

at 80°C for 2 hours in a sealed pressure tube. Upon removal of ethyl acetate under reduced pressure, the adduct was obtained in high yield as an oil (85:15 *exo-endo* ratio) and used without further purification. Mono protected diamine[24] **14** (6.8 g, 42.3 mmol) was added to the Diels-Alder adduct (6.1 g, 23.5 mmol) in DMAc (N,N-Dimethylacetamide, 6 mL) at 60°C and stirred for 20 minutes. A catalytic amount of cobalt acetate (0.5 mmol, 88.5 mg) dissolved in DMAc was added to this mixture followed by the addition of acetic anhydride (25 mmol, 255 mg) and the reaction mixture was stirred for 4 hours at 80°C. After cooling to room temperature the solution was diluted with ethyl acetate, washed with water and dilute HCl (5%), dried, and evaporated under reduced pressure to afford 95% yield of **12** with a 87:13 *exo-endo* ratio. Recrystallization from cold diethyl ether afforded pure *exo* isomer **12** (50%). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.42 (2H, t, *J*=2.1 Hz), 5.05 (1H, s), 3.70 (2H, t, *J*=1.9 Hz), 3.53 (2H, t, *J*=5.4 Hz), 3.25 (2H, broad d, *J*=5.0 Hz), 2.75 (2H, s), 1.82 (4H, t, *J*=7.8 Hz), 1.42 (9H, s), 1.22 (4H, m), 0.81 (6H, t, *J*=7.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 177.6, 155.8, 141.9, 137.8, 123.2, 78.9, 47.8, 45.1, 38.8, 38.4, 33.1, 28.2, 21.7, 13.9. Fast atom bombardment (FAB) HRMS calcd for $C_{23}H_{35}N_2O_4$ 403.260 g/mol, found 403.260 g/mol.

3.2.7 Preparation of 13

The same procedure that was used for the preparation of **9** from **8** was used for the preparation of **13** from *exo*-7-oxanorbornene-2,3-dicarboxylic anhydride[26] to afford 86% yield of **13** as the pure *exo* isomer. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.52 (2H, s), 5.27 (2H, s), 4.82 (1H, s), 3.64 (2H, t, *J* = 5.6 Hz), 3.30 (2H, dt, *J* = 10.9 Hz, 5.3 Hz), 2.86 (2H, s), 1.42 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 176.5, 156.1, 136.6,

81.1, 79.5, 47.5, 38.9, 38.6, 28.5. Repeated elemental analyses resulted in low carbon content. Elemental analysis for $C_{15}H_{20}N_2O_5$ (308.34 g/mol) calculated: C, 58.43; H, 6.54; N, 9,09. Found: C, 57.29, 57.23; H, 6.54, 6.52; N, 9.10, 9.10. HRMS (EI) calcd for $C_{15}H_{21}N_2O_5$ 309.145 g/mol, found 309.260 g/mol.

3.2.8 Polymerization of 8

The polymerization of **8** is a representative procedure for all other monomers, exceptions will be noted. Catalyst **4** was used for the polymerizations of **8**. A solution of catalyst was added to a dichloromethane (0.5 mL) solution of **8** (0.3 mmol, 61 mg) at room temperature, under an inert atmosphere. Catalyst to monomer molar ratios ranging from 1/10 (0.03 mmol catalyst) to 1/50 (0.006 mmol catalyst) were employed depending on the targeted molecular weight. The mixture was allowed to react for 0.5 to 1 hour depending on the catalyst to monomer ratio during which precipitation of poly**8** was observed. Ethyl vinyl ether (0.2 mL) was added and the precipitated solid was filtered and washed with pentane. The polymers were dried overnight under reduced pressure at room temperature. The isolated yields were between 88 and 90% (54-55 mg). A small sample was used for molecular weight determination using DMF GPC, relative to poly(ethylene oxide) standards (Table 3.1). Polymers were further characterized after the hydrolysis of anhydride group (Section 3.2.16), using aqueous GPC, again relative to poly(ethylene oxide) standards (Table 3.1). ^{1}H NMR (300 MHz, d-DMSO, ppm): δ 5.60-5.10 (2H, br), 3.69 (2H, br), 3.46 (2H, br), 1.66 (6H, s). ^{13}C NMR (75 MHz, d-DMSO, ppm): δ 173.9, 134.3 (br), 132.2 (br), 131.0 (br), 130.3 (br), 51.8 (br), 49.3, 48.9, 47.8, 21.1.

3.2.9 Polymerization of 9

Catalyst **4** to monomer molar ratios ranging from 1/5 to 1/45 were employed. The polymerization was terminated by addition of ethyl vinyl ether (0.2 mL) followed by precipitation in 10 mL of pentane. The isolated yields were between 85 and 90%. A small sample was used for molecular weight determination using THF GPC, relative to polystyrene standards (Table 3.1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 5.60-5.24 (2H, trans, br), 5.22-4.80 (2H, cis, br), 3.66 (2H, s), 3.57 (2H, s), 3.26 (2H, s), 3.08 (2H, s), 1.67 (6H, s), 1.40 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 178.9, 155.7, 135.1 (br), 131.2 (br), 130.0 (br), 79.1, 51.9, 50.9, 48.1, 43.7, 38.4 (br), 28.0, 21.1.

3.2.10 Polymerization of 10

Polymerizations of **10** were carried out using catalysts **3** and **4**. When catalyst **3** was used polymerization solutions were heated to 40-50°C. The isolated yields were between 90 and 94%. A small sample was used for molecular weight determination using THF GPC, relative to polystyrene standards. For the sample prepared by catalyst **3**, the M_n value was determined to be 22,000 g/mol, with a PDI value of 2.08. For the sample prepared by catalyst **3** the results are listed in Table 3.1. ^{1}H NMR (300 MHz, d-DMSO, ppm): δ 5.60-5.00 (2H, br), 4.00-3.20 (5H, br), 2.65-2.30 (1H, br), 0.84 (6H, s). ^{13}C NMR (75 MHz, d-DMSO, ppm): δ 173.2 (br), 140.34 (br), 137.8 (br), 134.1 (br), 132.8 (br), 52.0 (br), 51.1 (br), 50.2 (br), 49.2 (br), 46.8 (br), 27.9, 23.2.

3.2.11 Polymerization of 11

Polymerizations of monomer **11** were carried out using catalysts **1**, **2**, **3**, and **4** (Figure 3.3). Dichloromethane was vacuum-distilled from CaH_2 for the polymerizations that employed catalyst **1**. Catalyst **2** was used in toluene solutions. Catalyst to monomer

molar ratios ranging from 1/5 to 1/150 were employed. In the case of catalysts **1**, **2**, and **3,** polymerization solutions were heated to 40-50˚C for 0.5 to 2 hours depending on the catalyst to monomer ratio. Catalyst **4** was used at room temperature. Polymerizations were stopped by the addition of 0.2 mL of ethyl vinyl ether for catalysts **2**, **3** and **4**, or 0.2 mL of benzaldehyde for catalyst **1**, followed by the precipitation of the polymer into pentane. The isolated yields were between 85 and 95%. A small sample was used for molecular weight determination using THF GPC, relative to polystyrene standards (Table 3.1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 5.70-5.30 (2H, trans, broad d, J = 51.6), 5.30-4.85 (2H, cis, br), 4.10-3.95 (broad s), 3.95-3.80 (broad s), 3.80-3.50 (broad s), 3.40-3.20 (broad s), 3.20-2.85 (broad s), 2.70-2.30 (1H, br), 1.37 (9H, s), 0.89 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 179.2, 156.1, 139.8 (br), 136.6 (br), 132.5 (br), 79.5, 51.8 (br), 50.2, 46.9 (br), 38.9 (br), 28.5, 27.0, 26.4, 23.2.

3.2.12 Polymerization of 12

Polymerizations of **12** were carried out using catalysts **2** and **4**. Catalyst **2** was used in toluene and polymerization solutions were heated to 50˚C for 30 minutes. The isolated yields were between 80 and 90%. A small sample was used for molecular weight determination using THF GPC, relative to polystyrene standards (Table 3.1). Polymers were further characterized using NMR spectroscopy after the deprotection of pendant primary amine groups (Section 3.2.16).

3.2.13 Polymerization of 13

Polymerizations of **13** were carried out using catalysts **3** and **4** at room temperature. The isolated yields were between 90 and 95%. A small sample was used for molecular weight determination using THF GPC, relative to polystyrene standards.

For the sample prepared by catalyst **3** M_n value was determined to be 22,000 g/mol, with a PDI value of 1.94. For the sample prepared by catalyst **4** M_n value was determined to be 10,250 g/mol, with a PDI value of 1.07. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 6.05 (trans, s), 5.78 (cis, s) (2H, cis/trans = 44/56), 5.19 (1H, s), 5.02 (2H, cis, s), 4.51 (2H, trans, s), 3.59 (2H, s), 3.32 (4H, s), 1.39 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 176.0, 156.3, 131.5 (br), 131.0 (br), 79.7, 53.5, 52.3, 39.2, 38.5, 28.5.

3.2.14 Preparation of random copolymers

The preparation of poly(**9**$_2$-*co*-**11**$_1$) (M_n=15,300g/mol) with a comonomer ratio of 2 to 1, for **9** and **11** respectively, will be described as a representative procedure for the preparation of random copolymers of **9** and **11.** Comonomer feed ratio and catalyst to monomer ratio were changed in order to obtain random copolymers with desired comonomer content and molecular weights. A mixture of **9** (0.58 mmol) and **11** (0.29 mmol) was dissolved in dichloromethane (1.5 mL) and a solution of catalyst **4** (0.015 mmol in 0.05 mL of dichloromethane) was added at room temperature, under an inert atmosphere. The mixture was allowed to react for 90 minutes at 40°C. The random progression of the copolymerization was monitored using *in situ* ^{1}H NMR analysis, by probing the disappearance rates of the peaks at 1.53 ppm from **9**, and 2.24 ppm from **11** in deuterated chloroform solutions. Polymerization was terminated by addition of ethyl vinyl ether (0.2 mL) followed by precipitation in pentane resulting in a white polymer precipitate and brown supernatant. The product was filtered and dried overnight under reduced pressure at room temperature. The isolated yields were between 85 and 95%. A small sample was used for molecular weight determination using THF GPC, relative to

polystyrene standards (Table 3.2). Polymers were further characterized using NMR spectroscopy after the deprotection of pendant primary amine groups (Section 3.2.16).

3.2.15 Preparation of Poly(12-*b*-13) block copolymer

The preparation of poly(**12**-*b*-**13**) with a 50/50 ratio of each block will be described as a representative procedure for the preparation of block copolymers of **12** and **13.** Comonomer feed ratio and catalyst to monomer ratio were changed in order to obtain block copolymers with desired block ratios and molecular weights. Dichloromethane solutions (total 1.5 mL) of **12** (0.44 mmol) and catalyst **4** (0.035 mmol) were mixed at room temperature under an inert atmosphere and allowed to react for 2 hours at 45˚C. The temperature of the reaction solution was then decreased to room temperature and a dichloromethane (0.5 mL) solution of **13** (0.44 mmol) was added and allowed to react for 45 minutes. Polymerization was terminated by addition of ethyl vinyl ether (0.2 mL) followed by precipitation in pentane resulting in a white color polymer precipitate and brown color supernatant. The product was filtered and dried overnight under reduced pressure at room temperature. A small sample was used for molecular weight determination using THF GPC, relative to polystyrene standards. M_n was determined to be 11,000 g/mol with a PDI value of 1.13. Polymers were further characterized using NMR spectroscopy after the deprotection of pendant primary amine groups (Section 3.2.16).

3.2.16 Deprotection of poly9, poly11, poly12, poly13, poly(9_x-*co*-11_y), and poly(12-*b*-13)

Polymers bearing *t*-BOC protected primary amine groups resulting from the synthetic procedures described earlier were deprotected by dissolution of 100 mg of polymer in 2 mL of trifluoroacetic acid, and stirring at 45˚C for 8 hours. Polymers were

recovered in high yield by evaporation of trifluoroacetic acid under reduced pressure and dissolution in water followed by freeze-drying overnight. Polymers with deprotected primary amine groups will be noted using the prefix "dep-" followed by the notation of parent *t*-BOC protected polymer.

Dep-Poly(**9**), 80-90% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.90-5.10 (2H, br), 4.00-3.60 (4H, br), 3.50-3.00 (4H, br), 1.70 (6H, s). ^{13}C NMR (75 MHz, D_2O, ppm): δ 181.3 (br), 163.7, 163.3, 162.8, 162.3, 134.9 (br), 131.5 (br), 130.6 (br), 122.6, 118.7, 114.9, 111.0, 52.9, 51.6 (br), 48.5 (br), 44.1, 37.8, 36.7, 21.1.

Dep-Poly(**11**), 80-90% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.90-5.05 (2H, br), 3.81 (4H, br), 3.20 (4H, br), 2.44 (1H, br), 0.87 (6H, br). ^{13}C NMR (75 MHz, D_2O, ppm): δ 180.8 (br), 163.5, 163.1, 162.6, 162.2, 139.5 (br), 136.0 (br), 132.2 (br), 122.8, 118.8, 114.9, 111.1, 51.6 (br), 50.1 (br), 46.5 (br), 37.8, 36.6, 29.0 (br), 22.6.

Dep-Poly(**12**), 75-90% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.70-5.20 (2H, br), 4.10-3.50 (4H, br), 3.40-3.05 (4H, br), 2.20-1.70 (4H, br), 1.55-1.10 (4H, br), 1.00-0.60 (6H, s). ^{13}C NMR (75 MHz, d-DMSO, ppm): δ 178.6 (br), 138.1 (br), 135.8, 132.4 (br), 51.3 (br), 47.9 (br), 44.2, 36.2, 33.5, 21.0, 13.8.

Dep-Poly(**13**), 85-95% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 6.08 (trans, s), 5.88 (cis, s) (2H, cis/trans = 44/56), 4.98 (2H, cis, s), 4.63 (2H, trans, s), 3.80 (2H, s), 3.61 (2H, s), 3.20 (2H, s). ^{13}C NMR (75 MHz, D_2O, ppm): δ 178.1, 163.9, 163.4, 162.9, 162.5, 132.2 (br), 122.8, 118.9, 115.1, 76.8, 53.3, 52.5.

Dep-Poly(**9**$_1$-*co*-**11**$_2$), 80-90% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.90-5.10 (2H, br), 4.35-3.55 (4H, br), 3.55-2.90 (4H, br), 2.65-2.30 (33% of 1H, br), 2.00-1.20 (66% of 6H, br), 1.10-0.60 (33% of 6H, br). ^{13}C NMR (75 MHz, D_2O, ppm): δ

180.4 (br), 163.7, 163.4, 163.2, 162.8, 162.3, 139.4 (br), 136.0 (br), 134.9 (br), 132. 2 (br), 131.4 (br), 130.6 (br), 122.6, 118.7, 114.9, 111.0, 52.8, 51.6 (br), 50.0 (br), 48.5 (br), 46.4 (br), 37.8, 36.7, 28.8 (br), 22.5, 21.0. ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.89 (trans), 5.69 (cis) (2H, cis/trans=47/53), 4.45 (2H, trans, s), 3.62 (2H, s), 3.43 (2H, s), 3.02 (2H, s).

Dep-Poly(**12**-*b*-**13**), 88% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.89 (trans), 5.69 (cis) (2H, cis/trans=47/53), 4.45 (2H, trans, s), 3.62 (2H, s), 3.43 (2H, s), 3.02 (2H, s). ^{1}H NMR (300 MHz, d-DMSO, ppm): δ 6.01 (trans, s), 5.80 (cis, s) (2H, cis/trans=44/56), 5.61 (2H, br), 4.93 (2H, cis, br), 4.53 (2H, trans, s), 4.30-3.50 (6H, br), 3.42 (2H, s), 3.30-2.70 (6H, br), 2.25-1.70 (4H, br), 1.60-1.15 (4H, br), 0.84 (6H, s). ^{13}C NMR (75 MHz, d-DMSO, ppm): δ. 178.6, 178.0, 163.8, 163.2, 162.7, 162.3, 138.1 (br), 135.8, 132.4 (br), 122.7, 118.7, 115.0, 76.7, 53.3, 52.4, 51.3 (br), 47.9 (br), 44.2, 36.2, 33.5, 21.0, 13.8.

3.2.17 Hydrolysis of poly8 and poly10

Polymers bearing anhydride groups were hydrolyzed by dissolution in aqueous solutions containing 0.5 to 1 M NaOH, equimolar to the total carboxyl groups in the polymer, followed by stirring for 3 hours at 50°C. The polymers were recovered either by lyophilization or by precipitation into DMF followed by centrifugation. The precipitated polymers were washed by THF and dried overnight under reduced pressure at room temperature.

Dep-Poly**8**, 80-90% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.41 (2H, broad s), 3.55 (2H, broad s), 2.82 (2H, broad s), 1.62 (2H, s). ^{13}C NMR (75 MHz, D_2O, ppm): δ 182.1, 137.9 (br), 132.8 (br), 127.5 (br), 57.2, 56.4, 49.5 (br), 46.2, 21.4.

Dep-Poly**10**, 75-85% yield, ^{1}H NMR (300 MHz, D_2O, ppm): δ 5.90-4.95 (2H, br), 3.90-3.20 (2H, br), 3.05-2.70 (2H, br), 2.65-2.25 (1H, br), 0.84 (6H, s). ^{13}C NMR (75 MHz, D_2O, ppm): δ 174.0 (br), 173.5 (br), 140.4 (br), 137.7 (br), 134.3 (br), 132.8 (br), 52.1 (br), 51.2 (br), 50.2 (br), 49.3 (br), 46.8 (br), 28.0, 23.3.

3.3 Results and Discussion

3.3.1 Monomer Synthesis

Fulvene derivatives were used as functionalized dienes for the Diels-Alder cycloaddition reaction with an appropriate dienophile to obtain the modular norbornene structures (Figure 3.5). Three different fulvene derivatives, 6,6'-dimethyl fulvene, 6-isopropyl fulvene, and 6,6'-di-n-propyl fulvene, were prepared through a simple synthetic methodology, pyrrolidine catalyzed condensation of cyclopentadiene with an aldehyde or ketone, resulting in high yields.[22] The hydrophobic character of the monomer and the resulting polymer can be tuned by the choice of fulvene derivative. The modular approach to the monomer preparation allows for a variety of different alkyl groups to be readily incorporated. This allows for facile increase, or decrease of the hydrophobic character of the monomer and thus the resultant polymer. At this point it is necessary to note that the attempted preparations of mono-n-alkyl substituted fulvene derivatives, namely n-butyl and n-pentyl fulvene, were not successful and resulted in a mixture of ill-defined waxy oligomeric products.

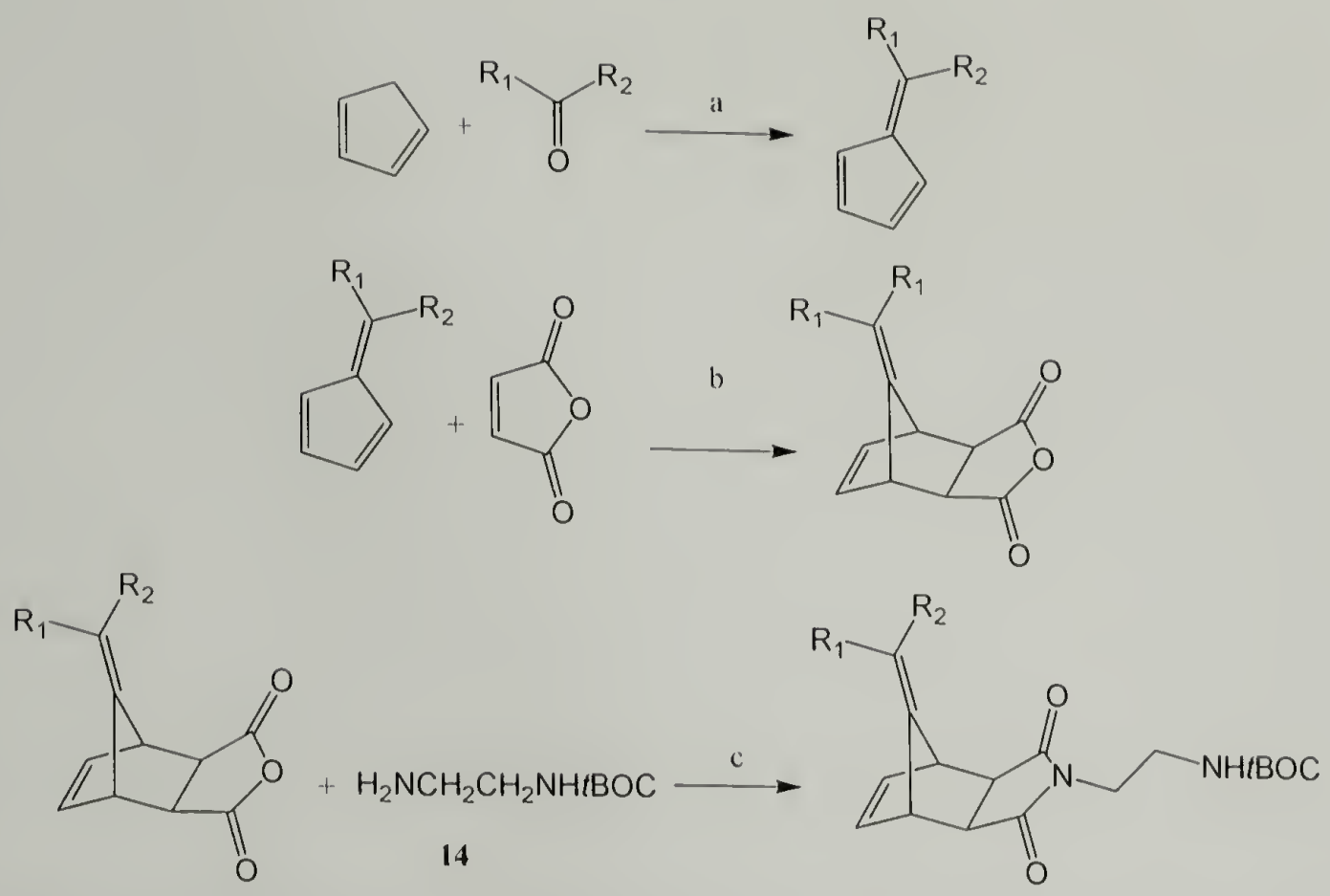


Figure 3.5 Representative preparation of modular norbornene derivatives. (a) ref. 22. (b) ref. 23. (c) $CoAc_2$, Ac_2O, DMAc, 80°C, 4 hours.

Maleic anhydride was used as the dienophile, allowing for further functionalization following the assembly of the norbornene skeleton. Diels-Alder cycloaddition of 6,6'-dimethyl fulvene, 6-isopropyl fulvene, or 6,6'-di-n-propyl fulvene to maleic anhydride at elevated temperatures, between 80°C and 120°C, and moderate concentrations, 0.2 to 0.5 M, afforded quantitative yields of the corresponding norbornene derivatives. At total adduct concentrations above 1.5 M or temperatures above 130°C a solid oligomeric side product, presumably a copolymer of the reactants, was obtained. When 6,6'-pentamethylene fulvene[22] was reacted with maleic anhydride the cycloaddition adduct was obtained in very low yield, and a white solid precipitated from ethyl acetate as the major product. The structure of which could not be determined using NMR analysis. As mentioned in section 3.1 two isomers, *endo* or *exo*, can be obtained from cycloaddition reactions, depending on the nature of adducts or the

reaction temperature. The two isomers of the monomer exhibit different polymerization kinetics, where in most case *endo* adducts polymerize very slowly, and result in low conversions. To achieve a high-level of control over polymerizations, and resulting polymer microstructures, the preparation of pure *exo* isomers of the monomers were targeted. When maleic anhydride was used as the dienophile *exo-endo* mixture of the cycloaddition adducts were obtained that were not always separable by selective recrystallizations. Although compounds **8**, **9**, **11**, and **12** were separated from their *endo* isomers through selective recrystallization to yield white crystalline solids, the *exo-endo* Diels-Alder adducts of 6-isopropylfulvene and maleic anhydride (**10**) could not be separated and remained as a brown oil. Cobalt catalyzed transformation of the anhydride into a substituted imide linkage resulted in the protected amine functionalized monomer structure in excellent yield. For both monomers **9**, **11** and **12** pure *exo* isomer was isolated by successive recrystallizations from cold ether, lowering the overall yield to between 40 to 56%.

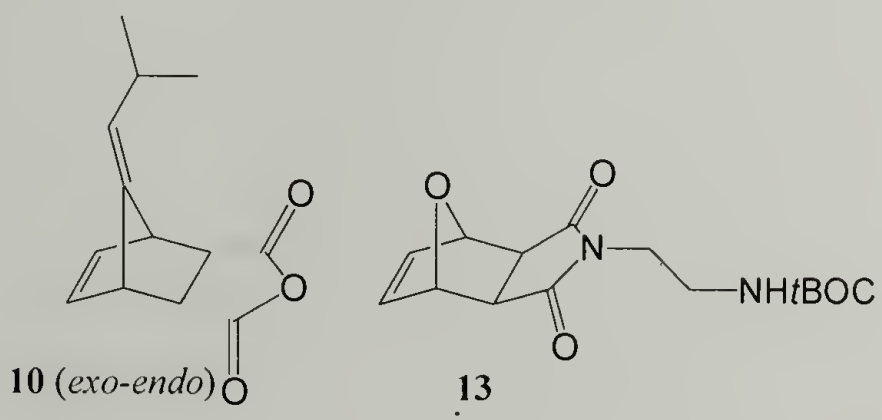


Figure 3.6 Compounds **10**, and **13**.

3.3.2 Homopolymerization Studies

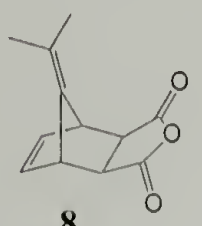
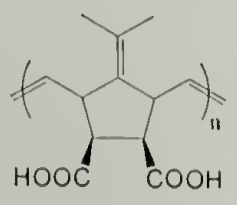
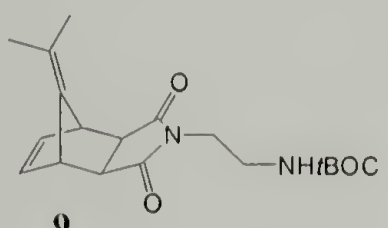
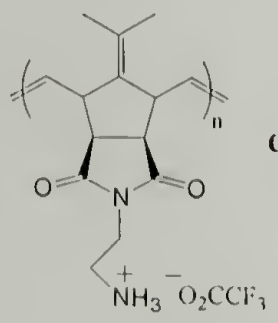
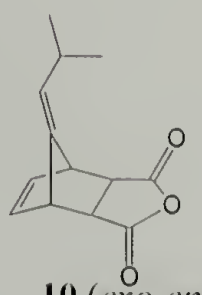
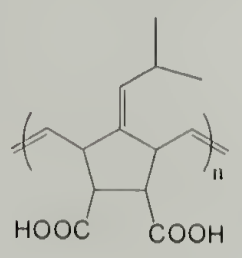
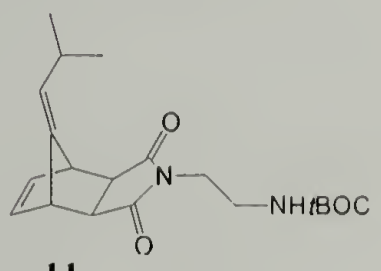
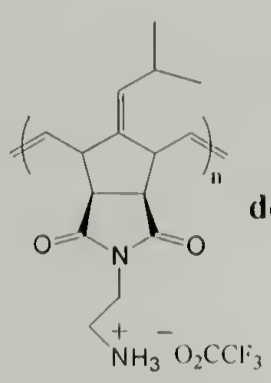
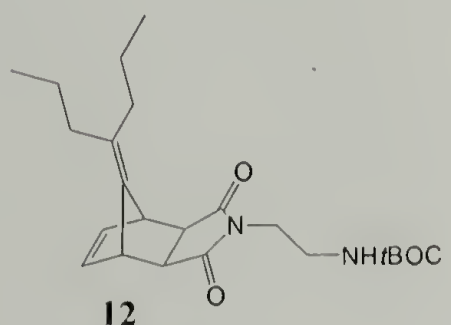
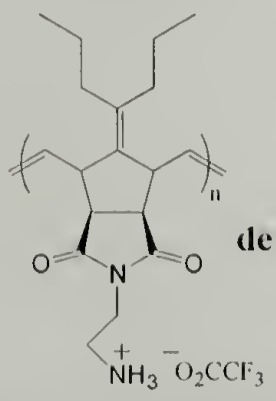
The initial target of the current study was to prepare amphiphilic polymers with well-defined architectures. Because the amphiphilic character was already dictated in the monomer unit, the target in the polymerization study of modular norbornene derivatives was to achieve controlled polymerization and obtain narrow polydispersities. The polymerization of a model monomer, **11**, was tested using four different metathesis catalysts, **1-4,** in order to screen the polymerizability and the effect of catalyst on the resulting polydispersities. The polymerization of **11** using catalysts **1-3** required elevated temperatures between 40-55˚C whereas catalyst **4** allowed for polymerization at room temperature. This result was in accordance with the reported high reactivity of catalyst **4**.[19] Desired molecular weights ranging between 1,600 g/mol to 75,000 g/mol (M_n) were obtained by adjusting the catalyst to monomer ratio for all four types of catalysts. For a targeted number average molecular weight of 8,800 g/mol at complete conversion, the polymerization of **11** using catalysts **1-4** resulted in polydispersity values of 1.23, 1.27, 1.96, and 1.10 respectively. Based on these results the homopolymerizations, and subsequent random and block copolymerizations, (Sections 3.3.3 and 3.3.4) involving monomers **8-12** were studied using catalyst **4** (Table 3.1). Poly**8** precipitated from the polymerization solution. Despite the early precipitation during polymerization, 88 to 90% yield of poly**8** was isolated with polydispersity values ranging between 1.14 and 1.17 (M_n ranging from 2,900 to 10,000 g/mol) . From the polymerization of monomer **9** using catalyst **4**, poly**9** was obtained in 85 to 90% yield with polydispersity values ranging between 1.08 and 1.13.

For all monomers the obtained molecular weights were in agreement with the targeted molecular weights as observed from GPC results. The slight discrepancy

between the targeted and observed moleeular weights of the polymers on Table 3.1 was expeeted due to the differences in hydrodynamie volume of these polymers versus narrow polydispersity polystyrene and poly(ethylene oxide) GPC standards. ^{1}H NMR end-group analysis was performed to eonfirm the mateh between the targeted and observed number average molecular weights for samples with M_n values less than 9000 g/mol. The relative integrations of the resonanees from the repeat units versus the multiplet from styrenic end-group at 7.32 ppm were in good agreement with the targeted molecular weight.

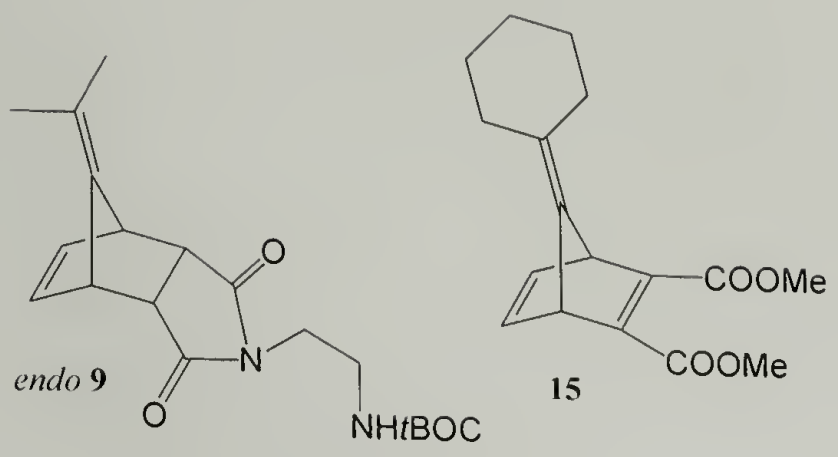
In a eontrol experiment the *endo* isomer of **9** was prepared. A proteeted amine functionalized maleimide derivative[25] was used as a dienophile for 6,6'-dimethyl fulvene at room temperature at a concentration of 0.4 M in ethyl acetate, for 2 hours. This protocol afforded an 14:86 *exo-endo* mixture of the monomer **9**, and pure *endo* isomer was obtained through reerystallization from diethyl ether. However, the *endo*-**9** monomer did not undergo ROMP at elevated temperatures and long reaetion times using eatalyst **3** or **4**. Presumably the combination of the alkylidene substituent at the seven position of the ring, and the *endo* 1,2-disubstitution resulted in enhanced steric hinderence, which precluded polymerization of the *endo* isomer.

Table 3.1 Examples of modular norbornene derivatives and amphiphilic polymers resulting from corresponding polymerizations[a] and deprotections.

Monomer	Deprotected Polymer	Theo. M_n[b] (g/mol)	Obs. M_n[c] (g/mol)	PDI[c]
 **8**	 **dep-poly8**	2,000 5,100 10,200	2,900[d] 7,000[d] 10,000[e]	1.15[d] 1.14[d] 1.17[e]
 **9**	 **dep-poly9**	2,400 5,900 19,800 29,900	1,950 7,000 17,900 24,100	1.13 1.08 1.11 1.13
 **10** (*exo-endo*)	 **dep-poly10**	10,900 21,900	9,500 19,500	1.63 1.49
 **11**	 **dep-poly11**	1,900 8,800 31,100 63,300	1,800 8,600 27,000 57,200	1.20 1.10 1.13 1.70
 **12**	 **dep-poly12**	4,900 14,600 32,300 60,500	5,300 14,500 32,200 57,000	1.09 1.24 1.13 1.19

[a] Polymers were prepared using catalyst **4**. [b] Theoretical molecular weights were calculated based on the catalyst to monomer ratio assuming full conversion. [c] Determined by THF GPC relative to polystyrene standards prior to the deprotection of polymer.[d] Determined by water GPC relative to poly(ethylene oxide) standards. [e] Determined by DMF GPC relative to polystyrene standards prior to the hydrolysis of polymer.

The polymerizations of *exo-endo* mixtures resulted in very low yields where only a fraction of the *exo* isomer was polymerized. Despite the presence of *endo* isomer in the case of monomer **10**, the *exo-endo* mixture was polymerized in good yields into high molecular weight polymers using catalysts **3** and **4,** however the resulting polydispersities were broader when compared to the other monomers (Table 3.1). In an attempt to eliminate the formation of *exo-endo* isomer mixtures and to increase the overall yields of the monomers, an electron deficient acetylene derivative, dimethylacetylenedicarboxylate, was used as the dienophile for the Diels-Alder reaction with 5,5'-hexamethylenefulvene to afford 2,3-dicarboxylic ester 7-alkylidene norbornadiene derivative **15**[27] in excellent yield (Figure 3.7). Preliminary studies showed that it was possible to convert the diester of **15** into anionic dicarboxylate groups by hydrolysis. From there a dicarboxylic anhydride was available by treatment with acetyl chloride and a protected primary amine can be obtained as side group by cobalt catalyzed attachment of **14** using the same procedure that was used for the preparation of monomers **9**, and **11-13**. However **15** did not undergo ROMP using catalysts **3** or **4** even at elevated temperatures up to 60°C. A similar monomer structure was previously reported to not undergo polymerization using a derivative of catalyst **1**.[28] These results confirm that the use of pure *exo* isomers of modular norbornene derivatives are needed to achieve high yields and high levels of control over molecular weights.



endo **9** **15**

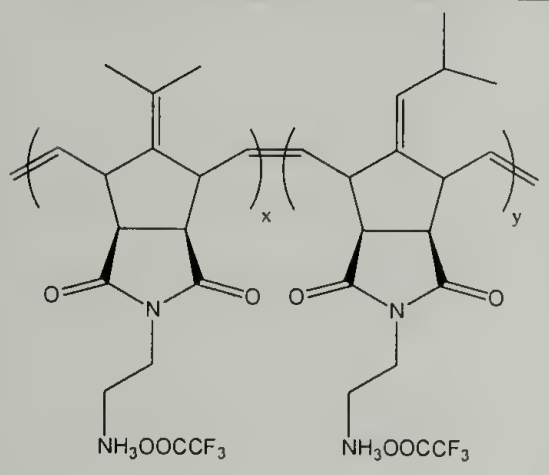
Figure 3.7 Compounds *endo* **9**, and **15**.

3.3.3 Random Copolymerization Studies

Previous sections of this chapter demonstrated the preparation of a series of novel homopolymers with amphiphilic character defined at the monomer unit. This approach has been shown to provide a controlled lateral amphiphilicity of a polymer with both functional characteristics, hydrophilic and hydrophobic, at the repeat unit. In the modular norbornene design a choice of ionic group, anionic or cationic, and a desired size of hydrophobic group can be predisposed for the polymer repeat unit. Although our demonstration has not gone further than having a hydrophobic 4-heptylidene group, on the 7 position of the norbornene ring, it is possible to increase the hydrophobicity to a much larger extent. As in the case of monomer **13**, where an oxygen atom replaces the alkylidene side group, it is also possible to eliminate the hydrophobic character of the monomer and thus also the resulting polymer. However, it must be noted that an extra carbon atom in the alkylidene side group could induce a large difference in the hydrophobicity of the repeat unit. For certain applications, as will be discussed in Chapters 4 and 5, a fine tuning of the hydrophobicity may be necessary. Copolymerizations of different modular norbornene derivatives provides a facile tool to further tune the overall hydrophilic/hydrophobic balance of the polymer without loosing

the lateral amphiphilicity of the repeat units. To demonstrate this concept copolymerization studies of monomers **9** and **11** provide an ideal model with respect to the very small difference in their hydrophobic side groups. Isopropylidene versus isobutylidene group has only one carbon atom difference. In order to obtain an overall hydrophobicity that would fall between homopolymers poly**9** and poly**11**, random copolymers, poly($\mathbf{9}_x$-*co*-$\mathbf{11}_y$)s, consisting of different comonomer ratios of **9** and **11** were prepared. As presented in Table 3.2 the subscripts x and y represent the relative comonomer content in the polymer.

Table 3.2 Examples of amphiphilic copolymers[a] of **9** and **11** resulting from corresponding copolymerizations and deprotections.

Copolymer Structure	Nomenclature	x/y (**9**/**11**)	M_n[b] (g/mol)	PDI[b]
	Poly($\mathbf{9}_9$-*co*-$\mathbf{11}_1$)	9/1	12,000	1.09
	Poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$)	2/1	15,300	1.15
		2/1	93,700	1.21
	Poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$)	1/2	8,500	1.09
		1/2	12,600	1.19
	Poly($\mathbf{9}_1$-*co*-$\mathbf{11}_4$)	1/4	11,800	1.15

[a] Polymers were prepared using catalyst **4**. [b] Determined by THF GPC relative to polystyrene standards prior to the deprotection of the copolymer.

Akin to homopolymerizations a large range of molecular weights were available without compromising narrow polydispersities. *In situ* ^{1}H NMR analysis revealed the equal disappearance rates of both monomers in all comonomer feed ratios reported. This data suggests a random copolymer formation, rather than a blocky polymer microstructure that would result from different polymerization rates. Because of the similarity of the two comonomers structures a detailed sequence analysis using NMR

speetroseopy, similar to the analysis in Chapter 2, could not be performed. However our findings in Chapter 2 suggest that it would be very unlikely to obtain a high level of alternating eharaeter from the eopolymerizations of **9** and **11**, whieh are strueturally very similar in the vieinity of the polymerization site. Beeause the polymerizations go to eompletion, the comonomer eontent in the polymer was in perfect accordance with the comonomer feed ratio. This was best determined after the deproteetion of the copolymer, by the ^{1}H NMR analysis of the resolved integrated areas rising from eaeh type of comonomer incorporated in the eopolymers (Figure 3.8). Resonanees at 1.65 ppm from **9**, and 0.85 ppm from **11** in ^{1}H NMR speetroscopy, provide good resolution to determine the comonomer eontent. This eopolymerization approaeh easily allows various compositions to be explored, and hence the hydrophobieity of the polymer to be fine-tuned. The preparations of copolymers with a eomonomer **9** eontent ranging from 20 mol% to 90 mol% were prepared.

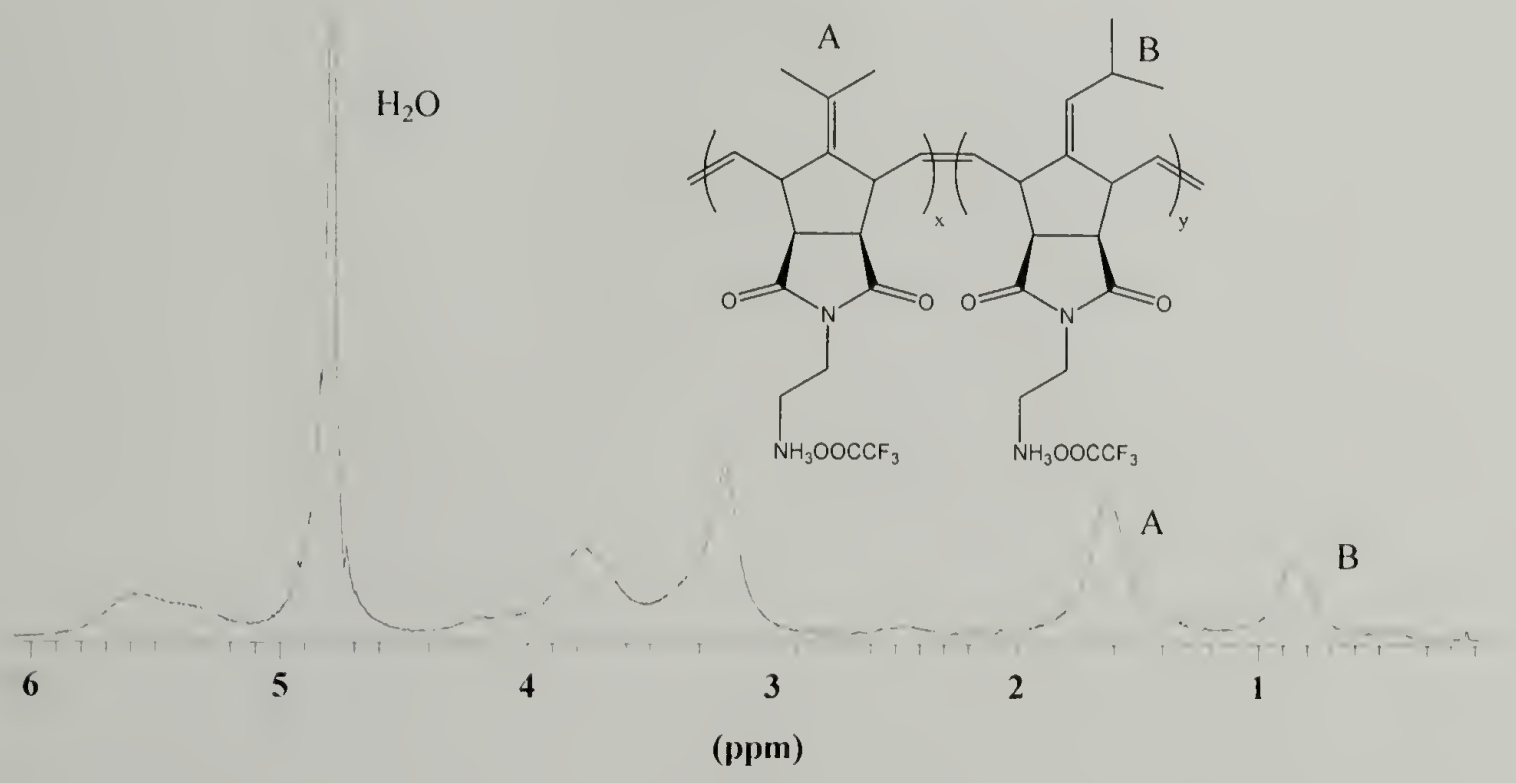


Figure 3.8 ^{1}H NMR spectrum of dep-poly(**9**$_2$-*co*-**11**$_1$) in D_2O. The ratios of the integrated areas for A and B is 2/1 respectively.

3.3.4 Block Copolymerization Studies

The narrow polydispersity values of the homopolymers and copolymers point to the living nature of **4** catalyzed ROMP of modular norbornene derivatives. Living polymerization systems provide access to block copolymers through sequential monomer additions into the active (living) chain ends.[29] Block copolymers, combining properties from both blocks, exhibit unique properties such as well-defined phase separations in the solid phase, or micelle formation in solvents that are selective for one block. As this chapter focuses on the preparation of amphiphilic polymers, it is relevant to note that water-soluble, micellar aggregates of amphiphilic block copolymers, consisting of hydrophobic and hydrophilic blocks, are promising materials for biomedical applications such as controlled drug delivery.[3,30,31] In principle, hydrophobic therapeutic agents that are insoluble in water, can be encapsulated in the hydrophobic core of the block copolymer aggregates and carried into aqueous media by the hydrophilic block. This section demonstrates the preparation of block copolymers with one block being an amphiphilic block based on modular norbornene derivatives. Therefore unique properties rising from the amphiphilic nature of the modular norbornene polymer, as will be discussed in Chapters 4 and 5, can be combined with advantages of block copolymer architectures. Block copolymerization of highly hydrophobic amphiphilic modular norbornene derivative **12** and highly hydrophilic **13** is demonstrated as a model system. Block copolymer with an equal molar ratio of poly**12** and poly**13** blocks were prepared by first generating a poly**12** block and addition of **13** to the second block. The complete consumption of **12** into a poly**12** block was confirmed using *in situ* ^{1}H NMR analysis, and monitoring the disappearance of monomer peaks. The GPC analysis of the initial poly**12** block and the resulting poly(**12**-

b-**13**) diblock copolymer in THF against polystyrene standards revealed a narrow monomodal molecular weight distribution, with an M_n value of 11,000 g/mol and a PDI value of 1.13, pointing to a well-controlled block copolymerization (Figure 3.9).

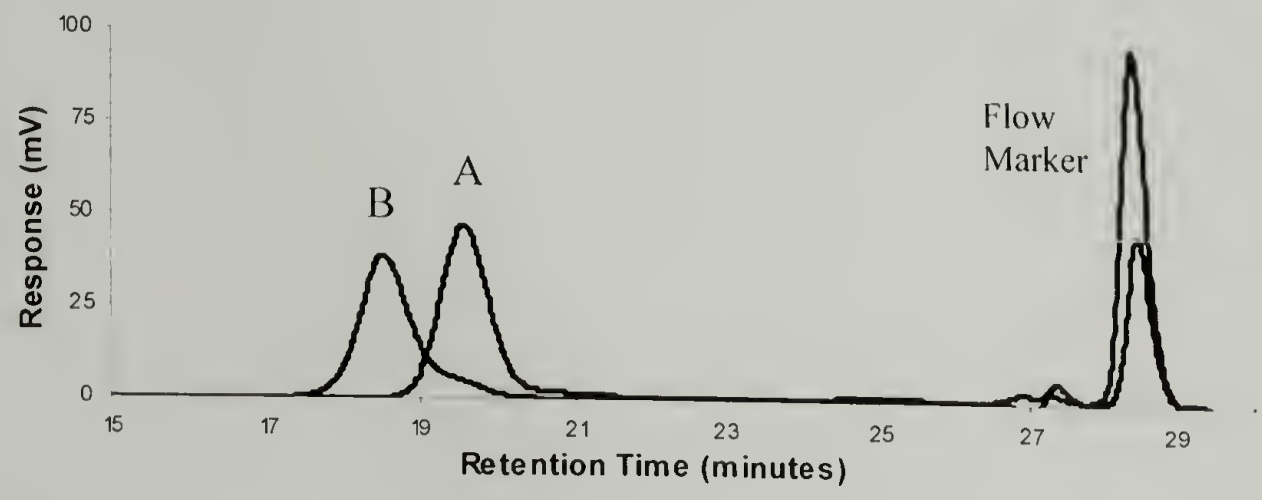


Figure 3.9 GPC traces of poly**12** block (A), and poly(**12**-*b*-**13**) diblock copolymer (B). Flow marker is toluene.

On the other hand, when a poly**13** was generated as the first block, the addition of **12** to the living poly**13** resulted in low conversions into second block and bimodal molecular weight distributions were observed from GPC analysis (Figure 3.10). Poor initiation of **12** from poly**13** could be possibly be due to the shielding effect of poly**13** coil around the propagating chain-end, preventing the sterically crowded monomer **12** to approach and polymerize from this polymeric macroinitiator. From this respect the efficient propagation from poly**12** macroinitiator could be suggested to be due to a more rod-like structure of highly crowded poly**12** backbone, better exposing the growing chain-end. This study demonstrated the conditions for the successful preparation of poly(**12**-*b*-**13**) block copolymer through sequential monomer addition, **12** then **13**.

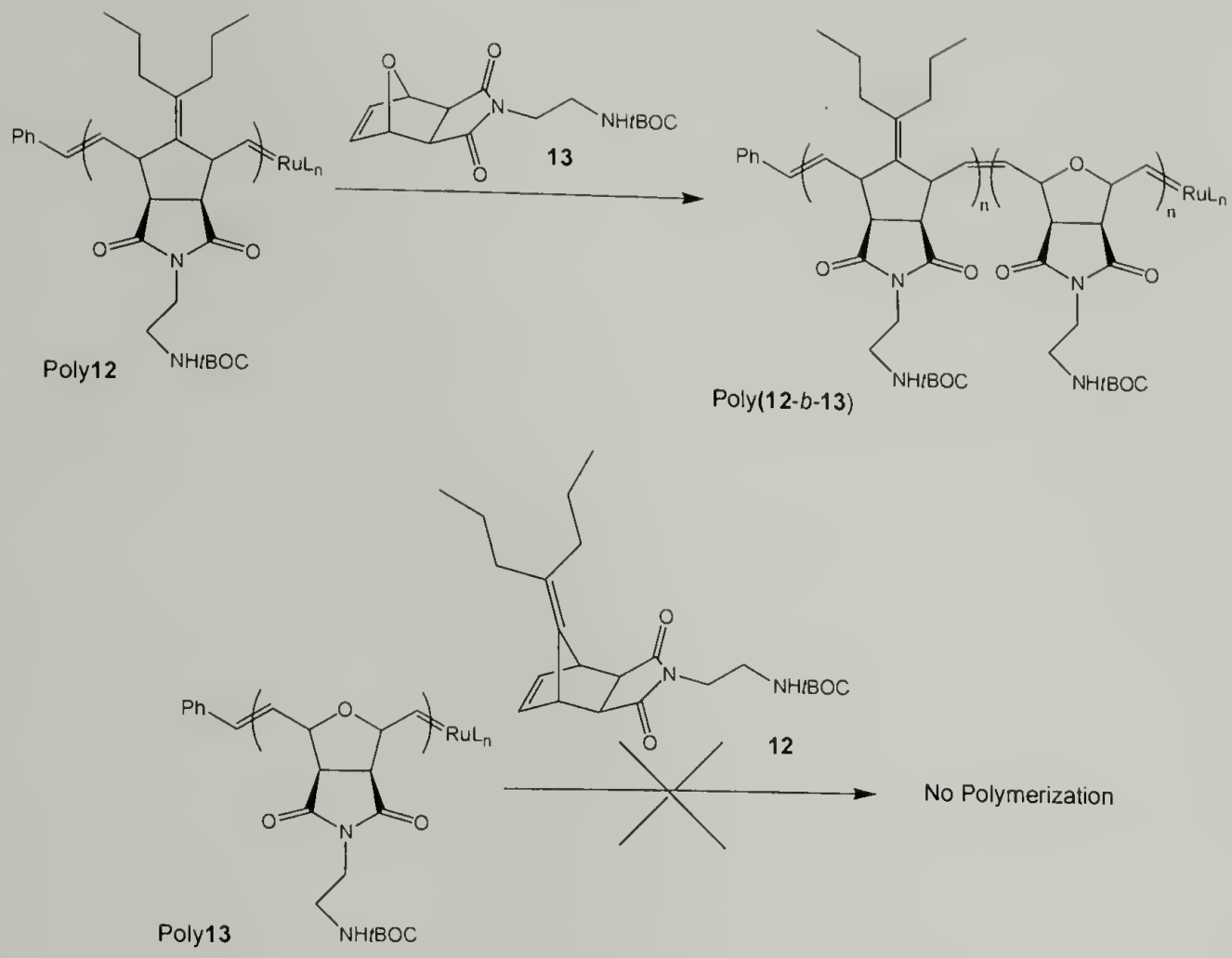


Figure 3.10 Sequential monomer additions for the block copolymerization of **12** and **13**.

3.3.5 Polymer Deprotections to Form Polyelectrolytes

The *t*-BOC protected pendant primary amine groups of poly**9**, poly**11**, poly**12**, poly**13**, poly($\mathbf{9}_x$-*co*-$\mathbf{11}_y$), and poly(**12**-*b*-**13**), and the anhydride functionalities of poly**8** and poly**10** provide a non-ionic and hydrophobic character to these polymers that allows for controlled ROMP, and subsequent characterization of the polymers in a wide range of organic solvents. To obtain the final amphiphilic nature of the polymers these groups were deprotected into their ionic forms resulting in water-soluble polymers. Protected primary amine functionalities of different molecular weight samples of polymers were deprotected quantitatively by dissolution in warm (45°C) trifluoroacetic acid (TFA) to obtain dep-poly**9**, dep-poly**11**, dep-poly**12**, dep-poly**13**, dep-poly($\mathbf{9}_x$-*co*-$\mathbf{11}_y$), and dep-poly(**12**-*block*-**13**) as observed by ^{1}H NMR recorded in D_2O solutions (Figure 3.11). ^{1}H

NMR spectra of these polymers also showed that carbon-carbon double bonds on the polymer backbone remain unaffected after treatment with TFA. Anhydride functionalities of poly**8**, and poly**10** were hydrolyzed successfully by dissolution of polymers in NaOH solutions to obtain dep-poly**8**, and dep-poly**10**. After these processes narrow polydispersity well-defined amphiphilic polymers with a desired anionic, or cationic, character and hydrophobic character were obtained. The importance of the structural variables of above mentioned homopolymers and random copolymers will be discussed in chapters 4 and 5.

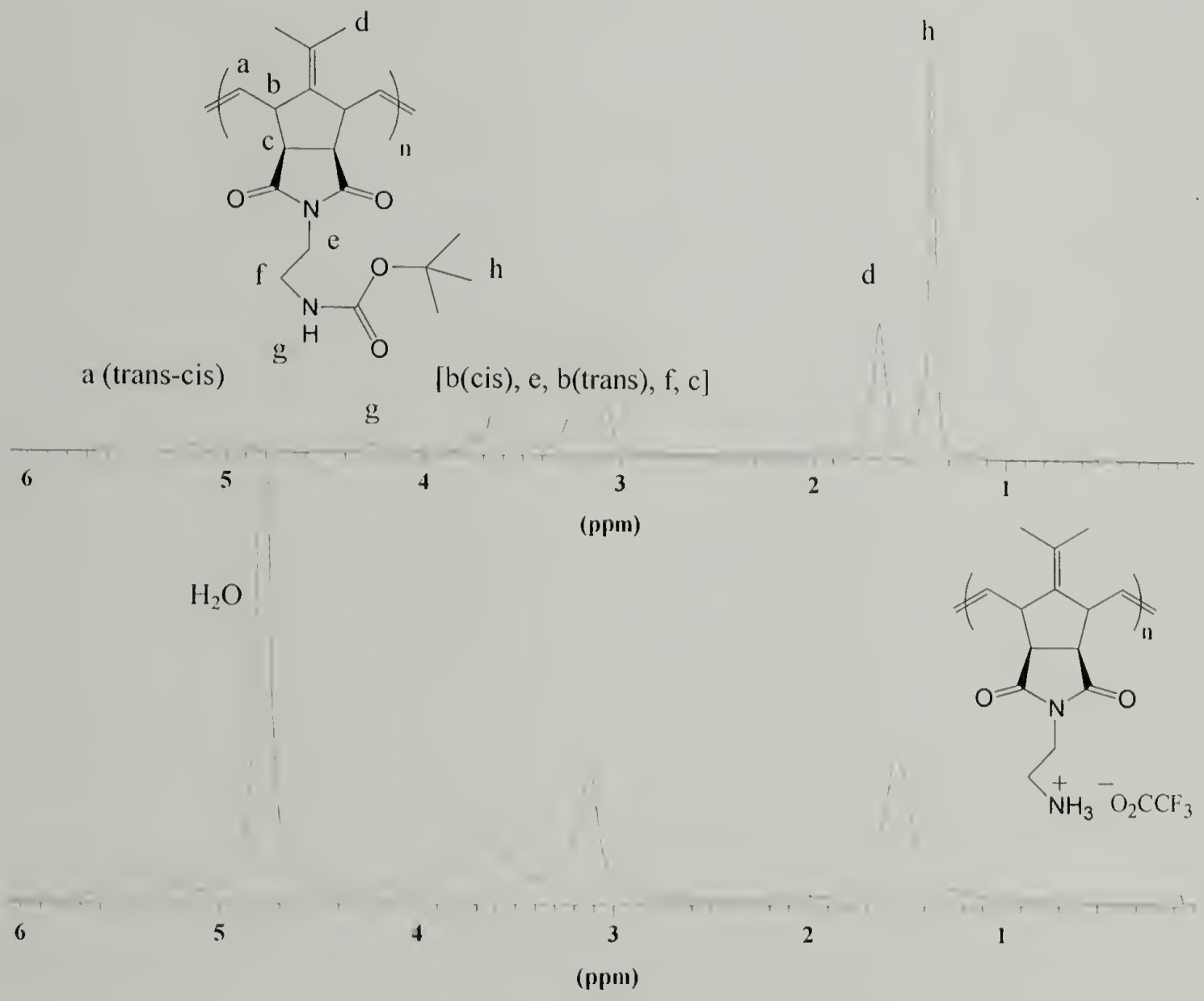


Figure 3.11 ^{1}H NMR spectra of poly**9** in $CDCl_3$ (top), and dep-poly**9** in D_2O (bottom).

The block copolymer, dep-poly(**12**-*b*-**13**), is a rather interesting polymeric architecture where one block is amphiphilic in itself, and both blocks carry charged groups. The strong hydrophobic character of the poly**12** block lowers its solubility in aqueous solutions. ^{1}H NMR spectroscopy of D_2O solutions of dep-poly(**12**-*b*-**13**), with a block molar ratio of 1/1, did not reveal the presence of poly**12** block, indicating an aggregate formation suppressing the mobility of the poly**12** (Figure 3.12). When a D_2O/DMSO-d_6 mixture or only DMSO-d_6 is used as solvent the resonances from hydrophobic block poly**12** were revealed. A core-shell structure is suggested, where more hydrophobic poly**12** is in the core.

The absence of the resonances from poly**12** block was confirmed at pH values between 2.5 and 7. On the other hand, the homopolymer of poly**12**, between molecular weights of 5,300 and 57,000 g/mol (M_n), is soluble in water below pH 6.6. Very limited solubility, below 1 mg/mL, or slow precipitation, was observed at or above pH 6.6. The aggregation of the block copolymer, dep-poly(**12**-*b*-**13**), at pH values below 6.6 could be due to the presence of highly hydrophilic poly**13** block, stimulating the hydrophobic interactions of 4-heptylidene side groups of poly**12** block. However here it can be suggested that pH dependent aggregate formation could potentially be obtained from modular norbornene based block copolymer systems by tuning the hydrophobic character and the block lengths. In addition potential biological activities rising from amphiphilic block, as will be discussed in Chapters 4 and 5, can be implemented into such aggregates.

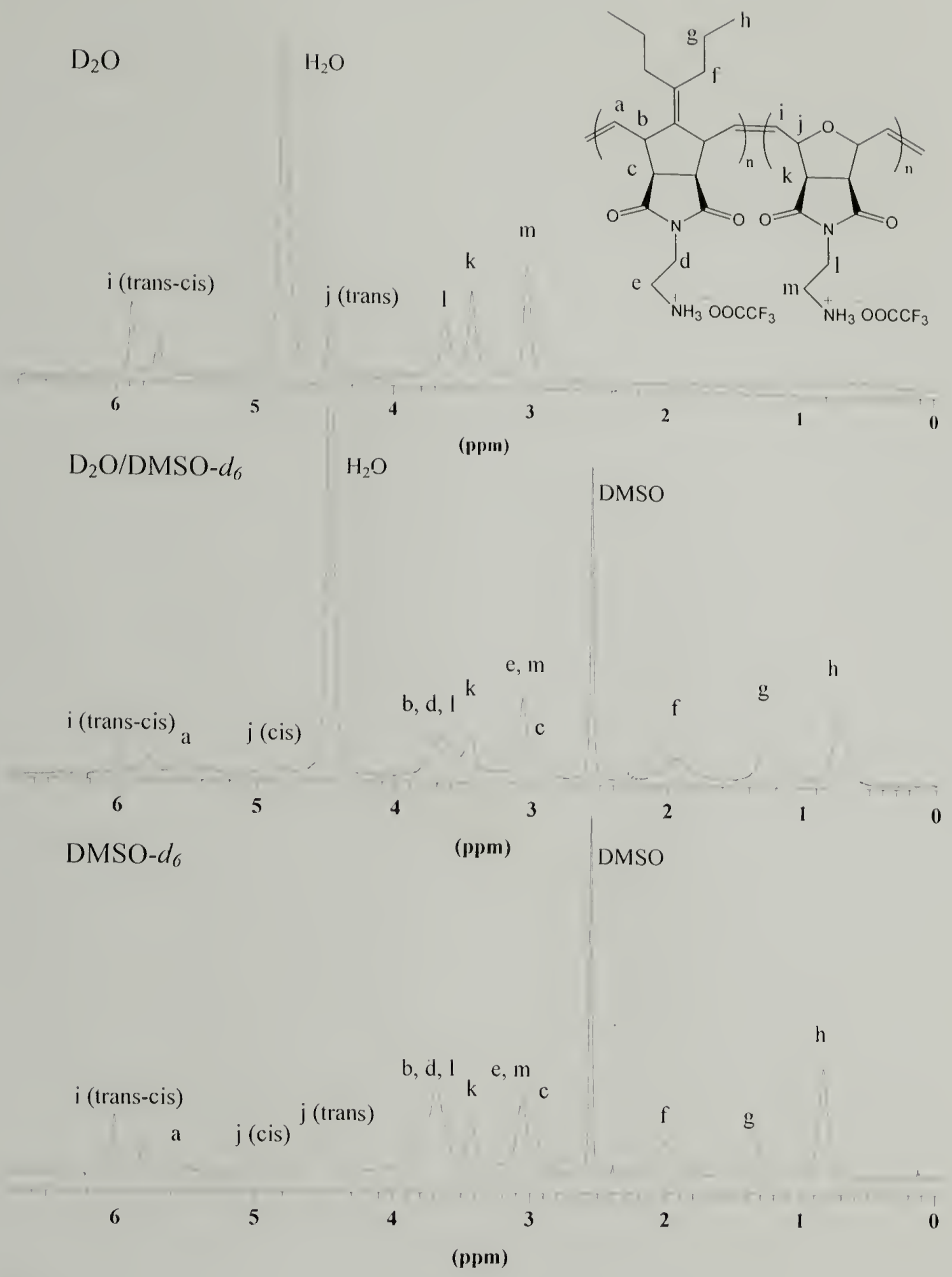


Figure 3.12 ^{1}H NMR spectra of poly(**12**-*b*-**13**) in D_2O (Top), 1/1 (v/v) D_2O/DMSO-d_6 (Middle), and DMSO-d_6 (Bottom).

3.4 Conclusions

In summary, synthesis and ROMP of modular norbornene derivatives possessing a dual character, hydrophilic and hydrophobic, have been developed and studied (Ilker, Schule and Coughlin, *Macromolecules*, **2004**, *37*, 694-700). This approach leads to polymers with lateral amphiphilicity, where the monomer has both domains. The synthesis of these modular monomers allows for the independent modification of the two regions of the monomer. In the case of the homopolymers, the amphiphilic character of the polymer, the hydrophilic/hydrophobic balance, is fixed on the repeat unit leading to a strictly uniform distribution along the backbone. This approach has been used for the preparation of novel amphiphilic polymers with a high level of structural control beginning at the repeating unit level, as well as control over polymer molecular weight, and polydispersity. The same strategy is extended to random copolymerizations of modular norbornene derivatives in order to fine-tune the overall hydrophobicity of the polymer. The preparation of block copolymers where one block is an amphiphilic block based on modular norbornene derivatives was also demonstrated. This approach is expected to combine the advantages of the controlled lateral amphiphilicity of modular norbornene based block with the unique properties expected from block copolymer architectures. The importance of structural control will be discussed in the next two chapters where the molecular weight and hydrophilic/hydrophobic balance of amphiphilic polymer will be shown to affect the interactions of the amphiphilic polymers with phospholipid membranes (liposomes), bacteria and human red blood cells.

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CHAPTER 4

STUDY OF PHOSPHOLIPID MEMBRANE DISRUPTION ACTIVITIES OF AMPHIPHILIC POLYMERS PREPARED VIA ROMP OF MODULAR NORBORNENE DERIVATIVES

4.1 Introduction

One interesting aspect of amphiphilic macromolecules is their interactions with phospholipid membranes, natural or artificial. Also amphiphilic in their chemical nature, phospholipid building blocks change their supramolecular ordering by incorporating amphiphilic polymers within their membrane assemblies. Depending on structural and compositional factors, various membrane deformations such as pore or tube formation, or complete disruption have been reported.[1-7] In this respect, biological activities of amphiphilic polymers are often associated with their ability to permeate cell membranes. Because phospholipid-based cell membranes are the principal structural components of living organisms, amphiphilic polymers and oligomers have attracted great research attention in the biomedical field. Applications, which are based on polymer induced transport through, or disruption of cell membranes, include drug delivery,[8-10] gene delivery[11-13] and antibacterial agents.[14-19] The antibacterial activity of cationic amphiphilic macromolecules, which is the major focus of the fifth chapter of this dissertation, has been suggested to be through perturbation of bacterial cell membranes.[1,20-22] Similarly, toxicity against mammalian cells can also be induced by the disruption of cell membranes, often measured as hemolytic activity against red blood cells.[17,19,23,24] The difference in the lipid contents of cell membranes from different organisms has been widely suggested to be one of the likely causes of the selective activities of certain membrane disrupting antibacterial agents.[1,14,23] Bacterial

cell membranes are known to contain an excess of negative charge on the polar outer surface of their cell membranes. Mammalian cell membranes on the other hand possess a neutral zwitterionic outer surface, and contain cholesterol that stiffens the membrane. The outcome from the exposure of phospholipid membranes to amphiphilic macromolecules is dictated by the detailed physiochemical properties of both parties.[23,25,26]

These scientific findings and suggestions, summarized above, were elucidated by a large number of studies that commonly utilize artificial liposomes, as model membranes.[1,7,19,25,27-31] Liposomes consist of a phospholipid bilayer envelope isolating an inner volume.[32,33] They are available through well-established preparative techniques that allow strict control over molecular components of the membrane and the environment. Depending on the preparation details the average diameter of vesicles typically change between 0.1-5 μm, with a lipid bilayer thickness of several nanometers. With these structural features liposomes have also been widely studied as microcapsules for drug and gene delivery applications.[34-37] Liposomes make it possible to monitor the dynamics of membrane perturbations, either by observing deformations using microscopy, if applicable, or by using an appropriate fluorescent dye encapsulated within, or excluded from, the liposome.[38-40] The leakage of the fluorescent dye across the membrane can be monitored as an indication of increased permeability or disruption of the membrane (Figure 4.1). In a typical experiment vesicles are loaded with a self-quenching concentration of fluorescent dye. Following the addition of membrane-disrupting agent, the disruption of vesicles can be monitored by quantitatively

measuring the increasing fluorescence arising from the leakage and dilution of the dye in the larger outer volume.

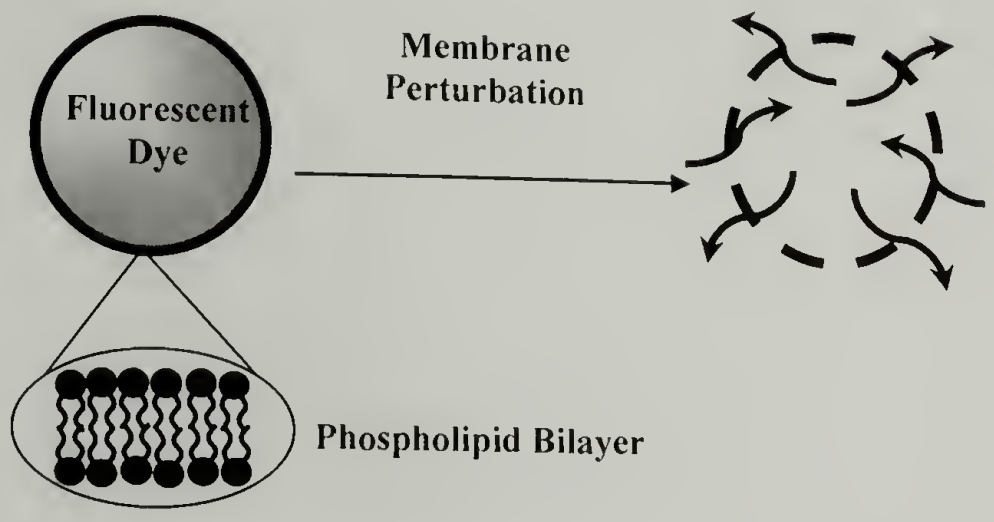
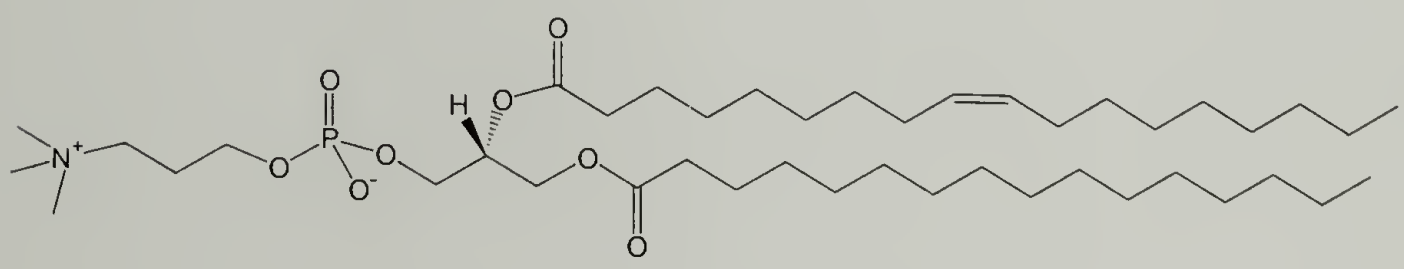


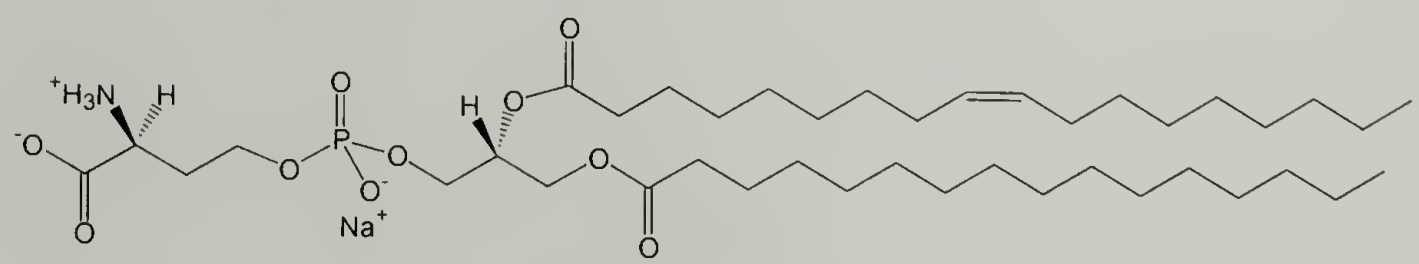
Figure 4.1 Representative illustrations of liposome and dye leakage experiment.

The disruption of neutral or anionic liposomes, with respect to their total lipid content and surface charge, have been commonly correlated to the selective activities of certain antibacterial agents against bacterial versus mammalian cells.[7,19,25,27-31] These assays are well documented in the literature and provide useful insight about the structure property relationships of membrane-disruptive agents.[17-19] Neutral zwitterionic liposomes, as mimics for mammalian cell membranes, are typically prepared from mixtures of phosphatidylcholine (SOPC) and cholesterol (CL) as a minor component (Figure 4.2).[19] Anionic liposomes on the other hand, are prepared from SOPC and anionic phospholipid phosphatidylserine (SOPS), as mimics for bacterial cell membranes.[1,6,7] These are simplified abiogenic models and therefore, in our study, these tests were used to evaluate the overall membrane disruption activities of polymers. We do not make direct comparisons of these results to activity against biological cells. Selective disruption activity against anionic liposomes or neutral liposomes often

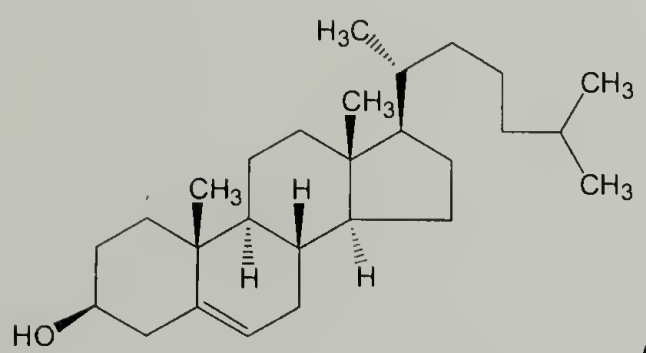
depends on very subtle structural details of the amphiphilic macromolecule. In this chapter the level of structural control over amphiphilic polynorbornene derivatives is used to control lipid membrane disruption activities. The effects of hydrophobicity and molecular weights of amphiphilic polymers, which were prepared as described in Chapter 3, will be probed against liposomes of different lipid content.



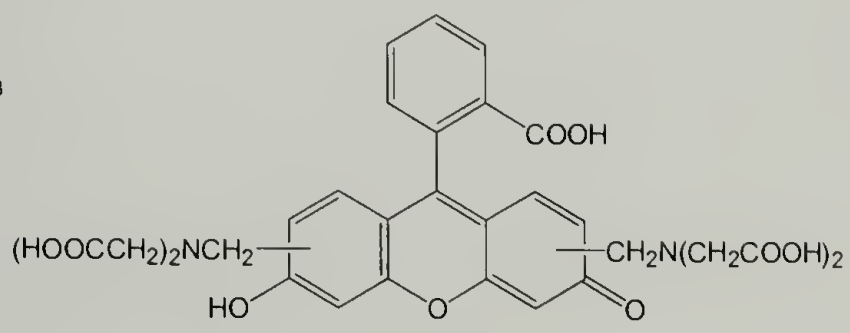
SOPC



SOPS



Cholesterol



Calcein

Figure 4.2 Structures of stearoyl-oleoyl-phosphatidylcholine (SOPC), stearoyl-oleoyl-phosphatidylserine (SOPS), cholesterol, and calcein used in the preparations of liposomes.

4.2 Experimental Section

4.2.1 Materials and Instrumentation

Stearoyl-oleoyl-phosphatidylcholine (SOPC, chicken-egg) and phosphatidylserine (SOPS, porcine brain-Na salt) were purchased from Avanti Polar-Lipids, Inc. Homopolymers dep-poly**8**, dep-poly**9**, dep-poly**10**, dep-poly**11**, dep-poly**12**, dep-poly**13**, and random copolymers dep-poly($\mathbf{9}_x$-*co*-$\mathbf{11}_y$)s were prepared as described in the experimental section of chapter 3. Reported molecular weights refer to values measured using THF GPC relative to narrow molecular weight polystyrene standards, prior to the deprotection of polymers into their water-soluble cationic forms. All other reagents were obtained from Aldrich. Fluorescence spectroscopy was recorded with a Perkin Elmer LS50B Luminescence Spectrometer.

4.2.2 Preparation of Liposomes (Lipid Vesicles)

4.2.2.1 Preparation of Anionic Liposomes

The anionic liposomes were prepared using a slight modification of literature procedures.[17,19,41] Chloroform solutions of SOPC (12.5 mg or 5 mg) and SOPS (1.5 mg or 7 mg) with molar ratios 9:1 or 1:1 were mixed. The chloroform was subsequently removed under a nitrogen stream followed by drying under reduced pressure for 3 hours at room temperature to obtain the lipid mixture as a dry film. The dried film was hydrated by addition of 2 mL of an aqueous solution containing calcein (40 mM) and sodium phosphate (10 mM, pH 7.0). The suspension was vortexed for 10 min. The suspension was sonicated three times, 7 minutes each, in a bath type sonicator (Aquasonic 150 HT) at room temperature. The suspension was freeze-thawed in acetone-dry ice and warm water baths after each sonication. This suspension was divided into two fractions, and one fraction was extruded through a polycarbonate filter

(Whatman, Nuclepore, 0.1 μm) 8 times using a double syringe extruder (Avanti Mini-Extruder). Both fractions were treated separately as follows. The non-trapped calcein was removed by eluting through a size exclusion Sephadex G-25-150 column with 90 mM sodium chloride, 10 mM sodium phosphate buffer (pH 7) as eluent. The average size of the non-extruded liposomes, which were prepared according to the above procedure, was determined to be 1 to 3 μm in diameter using optical microscopy (ZEISS Axiovert S100TV).

4.2.2.2 Preparation of Neutral Liposomes

The lipid vesicles were prepared using a slight modification of literature procedures[17,19,41] as described above (4.2.2.1) with the exception being that the initial mixture also contained cholesterol (1.7 μmol) that was dissolved in a chloroform solution of SOPC (17.2 μmol).

4.2.3 Determination of Polymer-Induced Leakage of Liposome Contents

Liposome suspensions, which were prepared as described above, were diluted 10-fold with the elution buffer prior to the addition of polymer. The polymer-induced leakage was monitored by recording the increase of calcein fluorescence intensity at 515 nm (excitation at 490 nm, slit width 3.0 nm). Phospholipid vesicles that were suspended in buffer solutions (pH 7) were stable and no increase of fluorescence was observed before the addition of the polymer. Complete liposome disruption was achieved by addition of 50 μl of 0.2 wt% TRITON-X 100, (polyoxyethylene(10) isooctylphenyl ether), a strong surfactant, after 3 minutes from the addition of the polymer, into the 3 mL suspension. The corresponding fluorescence intensity was then

taken to be equal to 100% leakage. The lysis caused by the polymer was reported as "% lysis" which is a fraction of the total lysis caused by TRITON-X 100.

4.3 Results and Discussion

4.3.1 Experimental Considerations

Before evaluating the results from the polymer induced dye leakage experiments it is appropriate to consider certain properties of liposomes that are dependent on the preparation details. Extrusion of liposomes through non-absorbing polycarbonate membranes, with pore sizes of 0.03 μm to 5 μm, is an effective method to reduce their size and increase the stability. Non-extruded liposomes are more likely to contain multilamellar vesicle (MLV) structures whereas extruded vesicles are richer in large unilamellar vesicles (LUV). Liposomes that were used in these studies were not extruded through polycarbonate filter except in the case of a control experiment involving dep-poly**11**, monitoring the stabilities of extruded and non-extruded liposomes against polymer induced membrane disruption (4.3.3). Although the preparation of each type of liposome population is well documented, the stability of phospholipid vesicle is dependent on their careful preparation. The preparation of liposomes is vulnerable to experimental errors. In each sub-study, the effect of different polymer concentrations, molecular weights, or hydrophobicity were compared using the same batch of liposomes in order to minimize the effect of experimental errors that could result from using different batches of liposomes. The same polymer samples, at the same concentration, were observed to induce different level of lyses in different batches of liposomes. Quantitative comparisons of the experimental results obtained from different batches is not possible, and our data evaluations take this factor into account.

4.3.2 Cationic Amphiphilic Polymers

Membrane disruption activities, and related biological activities, have been shown to be most commonly associated with the cationic amphiphilic nature of oligomeric or polymeric macromolecules.[42,43] Therefore our initial studies focus on the cationic amphiphilic polynorbornene derivatives, dep-poly**9**, dep-poly**11**, and dep-poly**12** (Figure 4.3). These polymers, being accessible across a range of molecular weights with narrow polydispersities, and with gradually increasing hydrophobicity from dep-poly**9** to dep-poly**12** on their side groups, provide an excellent model for studying the effect of molecular weight and polymer hydrophobicity on the lipid membrane disruption activities.

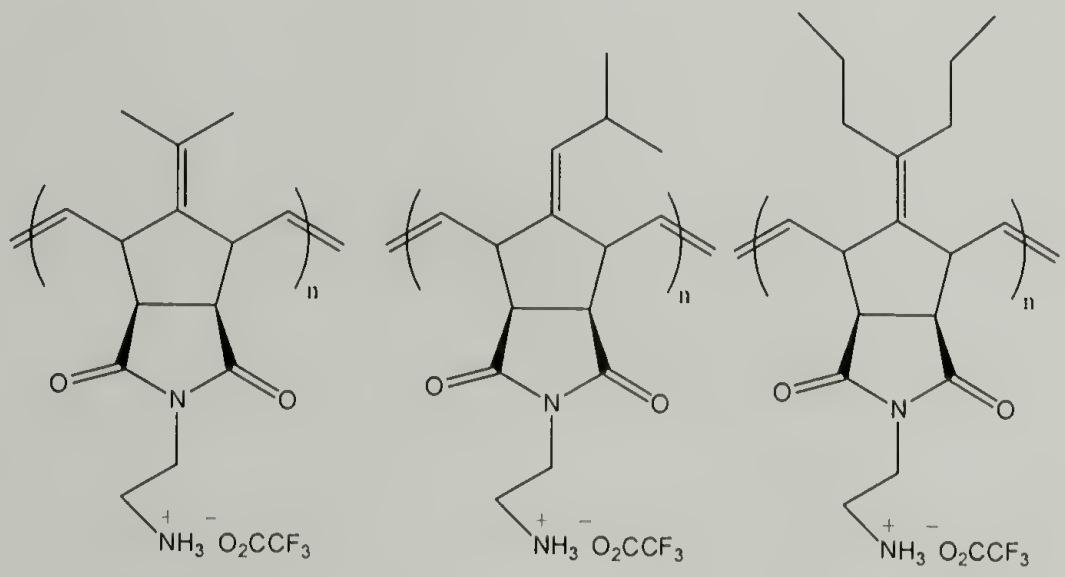


Figure 4.3 Amphiphilic polynorbornene derivatives with different hydrophobicity.

These studies were conducted side by side with the synthetic efforts presented in Chapter 3. Throughout our synthetic investigations, the first available amphiphilic polymer dep-poly**11**, which possesses an intermediate hydrophobicity compared to dep-poly**9** and dep-poly**12**, was also the first series to be probed for its membrane disruption activities against a series of neutral and anionic liposomes. Therefore the effects of lipid

eontent of liposomes, and polymer eoneentrations and molecular weights on the outeome of membrane disruption activities were elueidated using dep-poly**11**. Then the effeet of polymer hydrophobicity was probed by testing dep-poly**9** and dep-poly**12**, with decreased and inereased hydrophobieity respeetively.

When anionie liposomes that were prepared from 1:9 (molar ratio) mixtures of phosphatidylserine (anionie), and phosphatidyleholine (zwitterionie) lipids were exposed to dep-poly**11**, a 13,500 g/mol (M_n) sample eaused 100% lysis at concentrations as low as 5 μg/mL. Figure 4.4 shows the increase of fluoreseenee from caleein release in the first 3 minutes after the polymer addition, marked as %lysis, indicating the disruption of vesicles caused by different concentrations of dep-poly**11**.

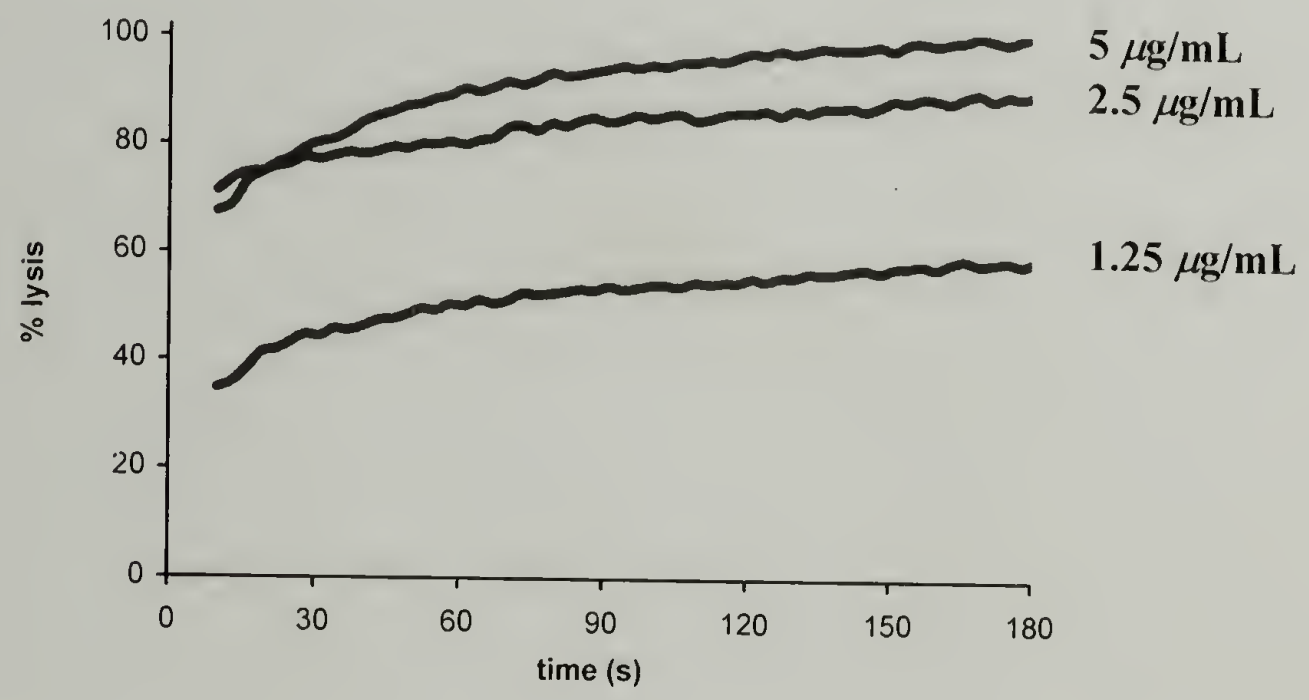


Figure 4.4 Lysis of anionie lipid vesicles in the presence of different coneentrations of dep-poly**11** (M_n = 13,500 g/mol).

Lysis was dose and molecular weight dependent (Figure 4.4 and 4.5). When a series of moleeular weights of dep-poly**11** ranging between monomer and 64,000 g/mol (M_n) were studied, it was observed that the membrane disruption aetivity was lower for the monomer and oligomers with molecular weights less than 4,500 g/mol. Dep-poly**11**

of molecular weights above 4,500 g/mol and up to 64,000 g/mol showed very high activities independent of molecular weight in this range. This result suggests that the membrane disruption activity of dep-poly**11** increases with molecular weight until it reaches a critical molecular weight necessary to obtain maximum membrane disruption activity. As described in Chapter 3, the living nature of ROMP allows for the precise targeting of the desired molecular weight and hence allows for tuning the membrane activity of dep-poly**11**s.

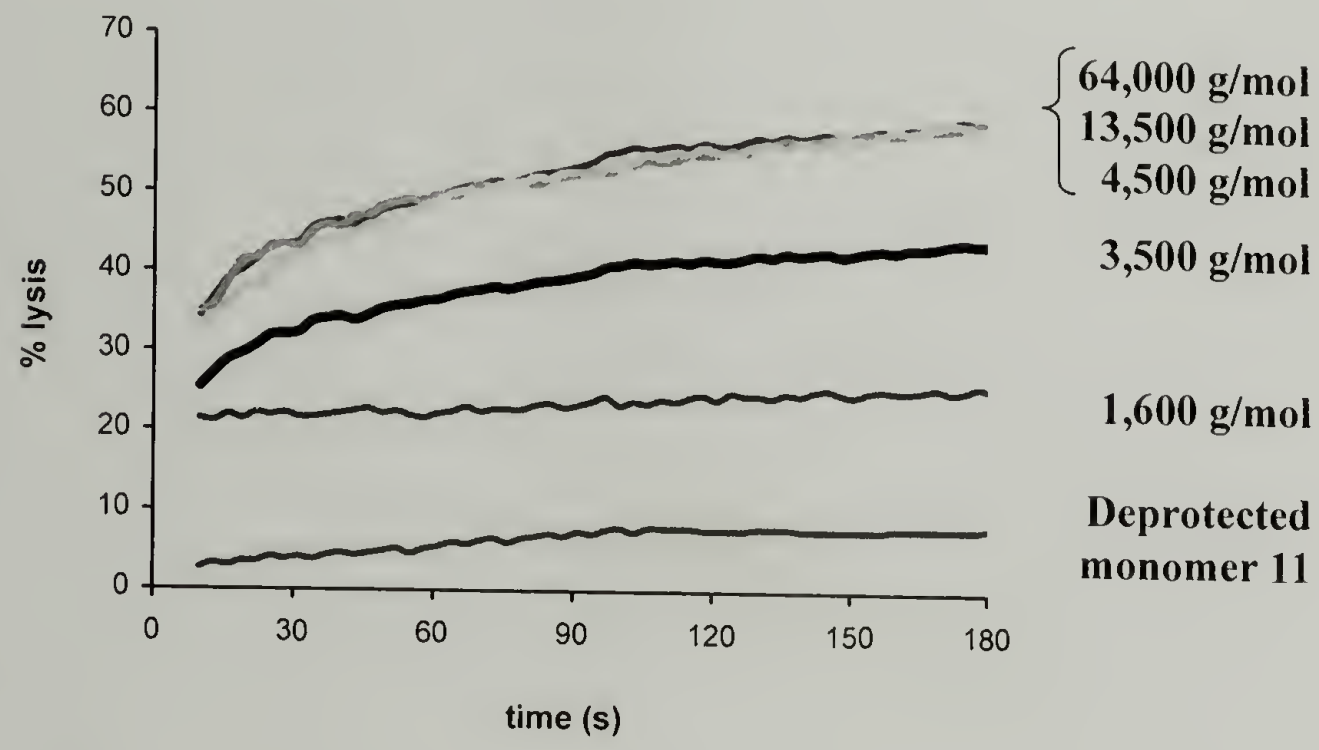


Figure 4.5 Lysis of anionic liposomes caused by different number average molecular weight (M_n) samples of dep-poly**11** at the concentration of 1.25μg/ml.

It should be noted that while probing the molecular weight effect, the concentration of the polymer added into the liposome suspension was calculated in terms of mass/volume. If the corresponding molar concentrations were to be calculated, the dep-poly**11** sample with a number average molecular weight of 64,000 g/mol would have 14 times fewer, but longer, chains than the dep-poly**11** sample of 4,500 g/mol at the same mass/volume concentration. With this idea in mind, very similar activities obtained from different molecular weights of dep-poly**11** at the same mass/volume

concentration may suggest a cooperative action of polymer chains being responsible for membrane disruption.

When the effect of molecular weight is probed against liposomes that were extruded through a polycarbonate membrane, the critical molecular weight for highest membrane disruption was shifted towards higher molecular weight values (Figure 4.6). It could be suggested that non-extruded liposome membranes are less stable allowing the lower molecular weight dep-poly**11**s to be more disruptive.

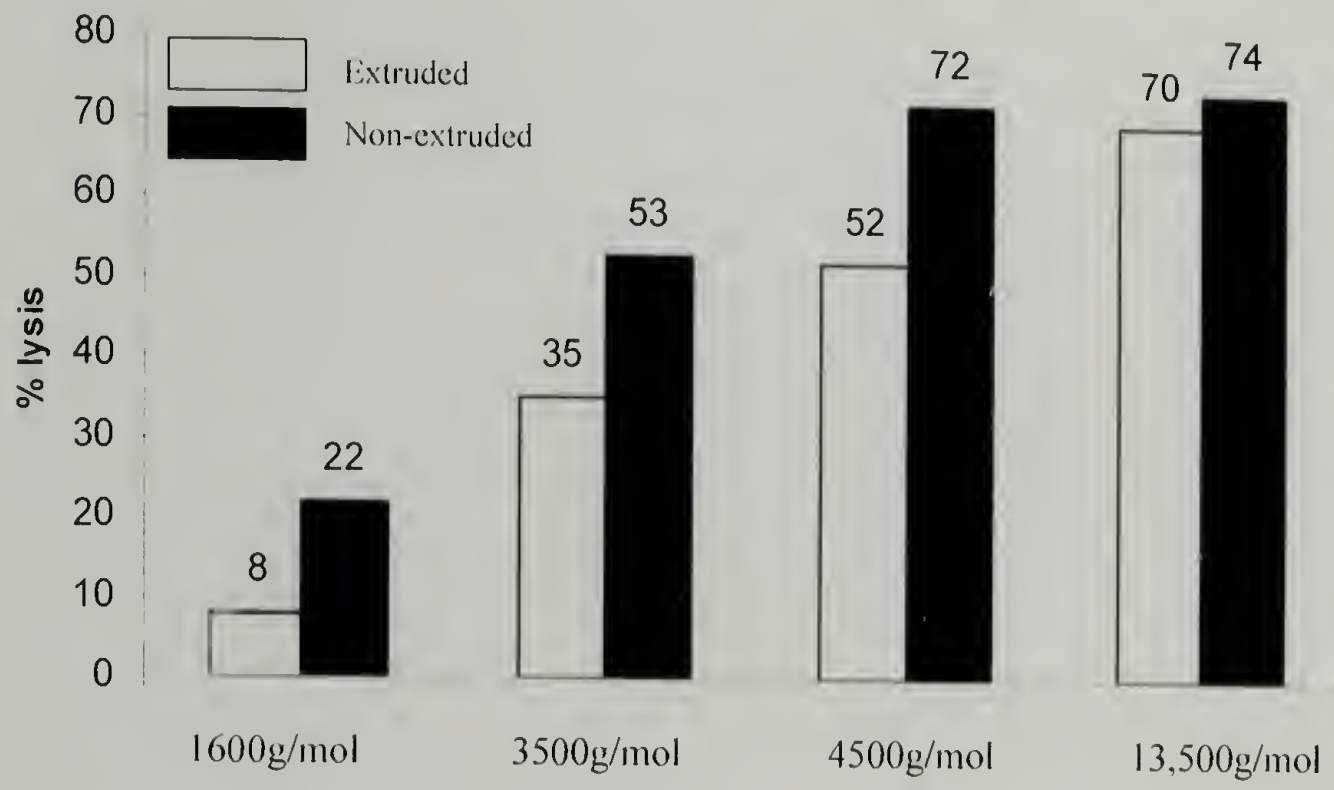


Figure 4.6 Lysis values at 3 minutes after the addition of dep-poly**11** of four different molecular weight into the liposomes that are extruded (empty bars) or not extruded (filled bars) through 0.1 μm polycarbonate filter.

The membrane activities of polymers were probed in liposome suspensions of pH 7, an approximate value for physiological pH, as we are ultimately targeting biological applications. Although an in-depth analysis was not performed, the pH of polymer stock solution, which was added in small quantities into larger volumes of liposome suspensions, was observed to have a remarkable effect on membrane

disruption activities. An approximately 2 to 10-fold increase in membrane disruption activities, depending on the molecular weight, was observed for polymer solutions at pH 3 compared to pH 6.5. The effect was more pronounced for lower molecular weight samples. A dep-poly**11** (M_n 1600 g/mol) solution of pH 3 was observed to cause a near 100% lysis as opposed to less than 15% lysis from a solution of pH 6.5 at the same concentration. A two to three fold increase in activity was observed for dep-poly**11** of 24,100 g/mol molecular weight (M_n). These are very preliminary results warranting a more detailed investigation of the effect of pH of polymer stock solutions. The data presented in this chapter was obtained using polymer solutions at pH values 6.5 to 7.

4.3.3 Effect of Membrane Composition of Liposomes

In order to observe the effect of lipid composition of the membranes on the activity of the dep-poly**11**, neutral, zwitterionic liposomes with a 9:1 SOPC to cholesterol molar ratio, and anionic vesicles with 9:1 and 1:1 SOPC to SOPS molar ratios were prepared. Batches of vesicles with different ionic character were tested within the same experiment, using the same reagents and equipment in order to minimize experimental errors. The results were also confirmed in a second set of experiment.

The stiffening effect of cholesterol on the membrane was revealed when liposomes were prepared in the absence of cholesterol in a control experiment. Neutral liposomes that did not contain any cholesterol but only SOPC were very unstable when compared to cholesterol or SOPS mixed liposomes. These liposomes resulted in early lysis, as observed from the initial high fluorescence.

When a solution of dep-poly**11** (M_n=27,000, PDI=1.13) in TRIS saline buffer (pH 6.5) was added to each of three different liposome suspensions, the membrane

disruption activity was observed to increase with increasing anionic lipid content of liposome from 0 mol% to 50 mol%. 20 μg/mL of dep-poly**11** caused 90% lysis in 3 minutes against anionic liposomes with 1:1 SOPC to SOPS ratio. The percent lysis values at the same experimental conditions decreased to 67% as anionic lipid content decreased to a 9:1 SOPC to SOPS ratio, and 24% for neutral vesicles with no anionic lipid content but 10% cholesterol content. These results show increasing affinity of dep-poly**11** for negatively charged liposome membranes. This trend is consistent with the cationic nature of the polymer causing stronger interactions between phospholipid membrane and the polymer. More than two fold selectivity against anionic liposomes can be induced by introducing 10% or more anionic lipid content.

4.3.4 Effect of Polymer Hydrophobicity

The previous sections have shown that high disruption activities against phospholipid membranes can be obtained from dep-poly**11** depending on polymer molecular weights and membrane composition. The activities of dep-poly**9** and dep-poly**12** with relatively lower and higher degrees of hydrophobicity were tested against neutral (SOPC: CL=9:1) and two different anionic (SOPC: SOPS=9:1 and 1:1) liposomes. Similar molecular weight samples of dep-poly**9** (M_n=24,100, PDI=1.10), dep-poly**11** (M_n=25,500, PDI=1.17), and dep-poly**12** (M_n=32,200, PDI=1.17) were compared within the same experiment (Figure 4.7). The membrane disruption activities of all polymers were observed to increase with increasing anionic strength of the membrane. However all three types of membranes were less vulnerable to both dep-poly**9** and dep-poly**12** when compared to dep-poly**11**. Increasing or decreasing the

hydrophobicity in reference to dep- poly**11** resulted in diminished membrane disruption activities.

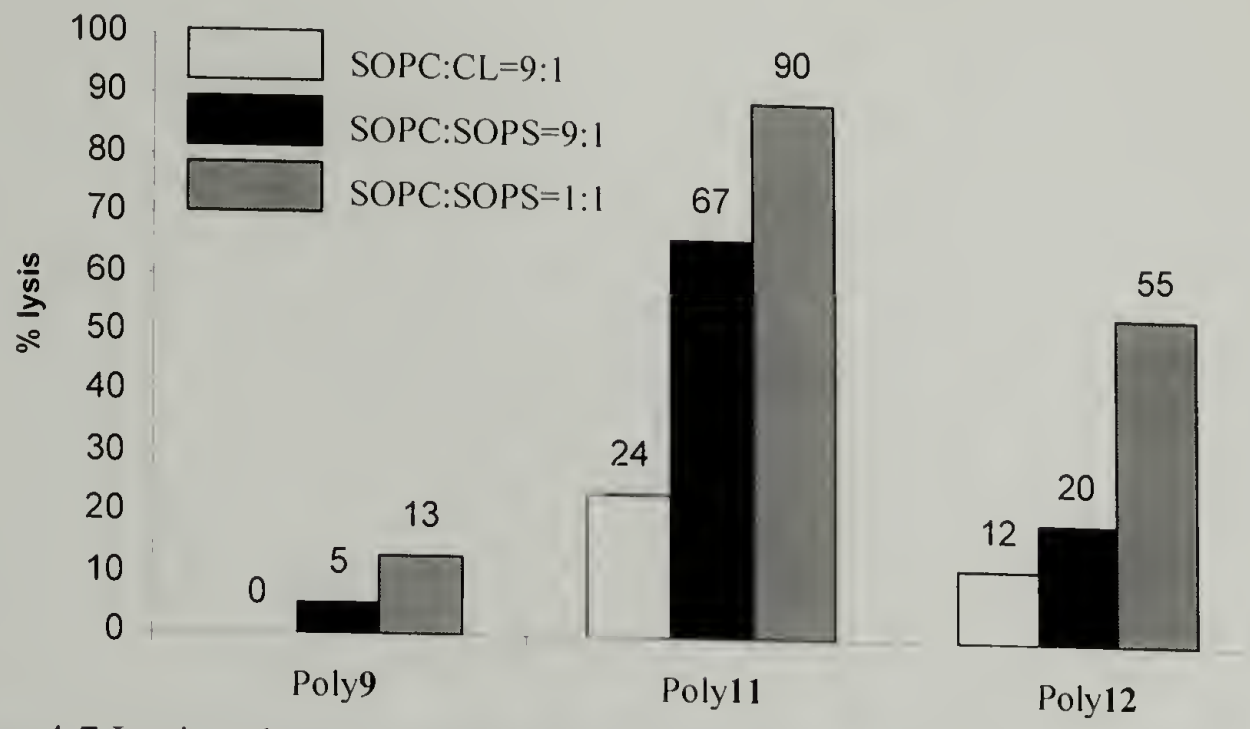


Figure 4.7 Lysis values 3 minutes after the addition of 40 μg/mL of dep-poly**9** (M_n=24,100, PDI=1.10), dep-poly**11** (M_n=25,500, PDI=1.17), and dep-poly**12** (M_n=32,200, PDI=1.17) into suspensions of neutral (left, SOPC: CL=9:1), anionic (middle, SOPC: SOPS=9:1, right, SOPC: SOPS=1:1) liposomes.

When the effect of molecular weight was probed for dep-poly**9** against anionic liposomes, increased molecular weights were shown to have increased activities, in accordance with the result obtained from dep-poly**11** (Figure 4.8). Finally when the hydrophobicity was totally removed, in the case of dep-poly**13** (M_n=25,000 g/mol), with an oxygen atom replacing the alkylidene group, the activity against anionic vesicles (SOPC: SOPS=9:1) was no more than 8% lysis, up to a sufficiently high polymer concentration, 200 μg/mL, within 3 minutes. These results reveal that a specific hydrophilic/hydrophobic balance is crucial to obtain highest membrane disruption activities from amphiphilic cationic polynorbornene derivatives.

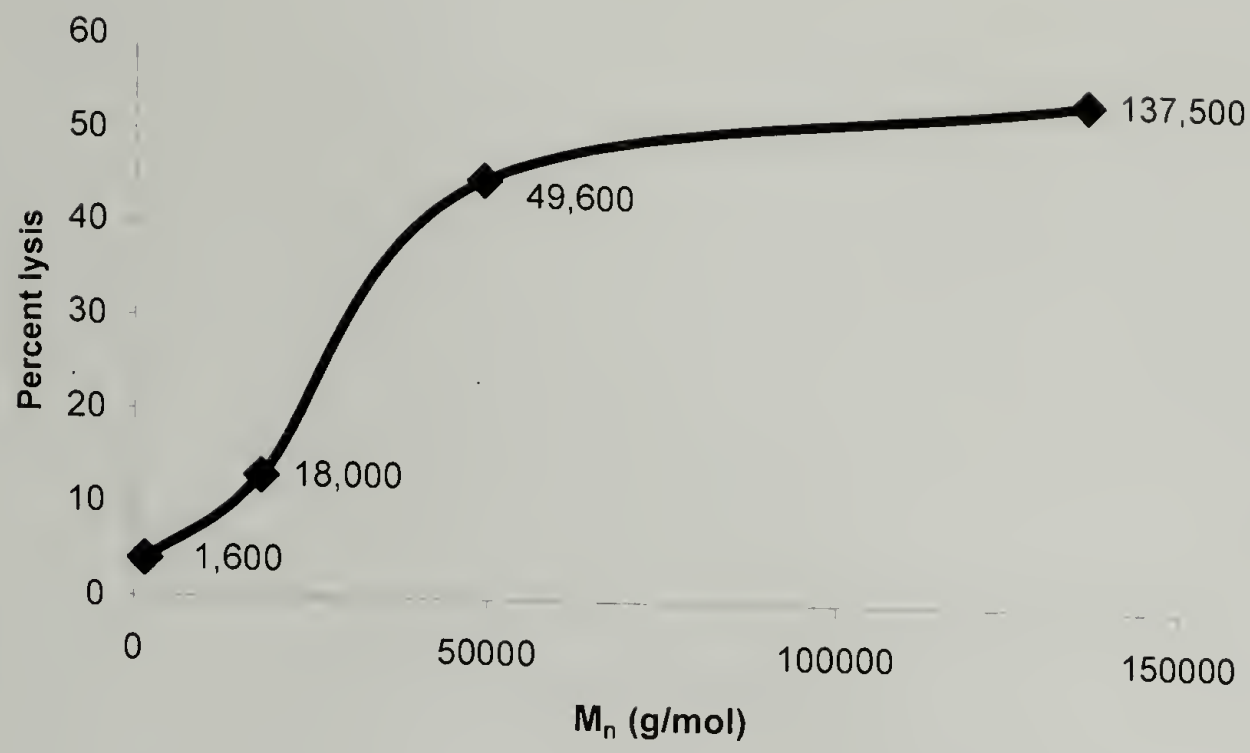


Figure 4.8 Percent lysis at 3 minutes after the addition of 40 μg/mL of four different molecular weights (M_n) of dep-poly**9**. Molecular weights are given next to data points.

4.3.5 Random Copolymers Poly($\mathbf{9}_x$-*co*-$\mathbf{11}_y$)

In chapter 3, random copolymerization of **9** and **11** has been shown to allow fine-tuning of the hydrophobicity of the amphiphilic copolymer. These random copolymers exhibit hydrophobicity intermediate between poly**9** and poly**11** homopolymers. The membrane disruption activities of dep-poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$) and dep-poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$), with final comonomer molar ratios of 2/1 and 1/2 respectively, were compared against similar molecular weights of dep-poly**9** and dep-poly**11** to establish a more detailed pattern of polymer hydrophobicity membrane disruption activity relationship (Figure 4.9).

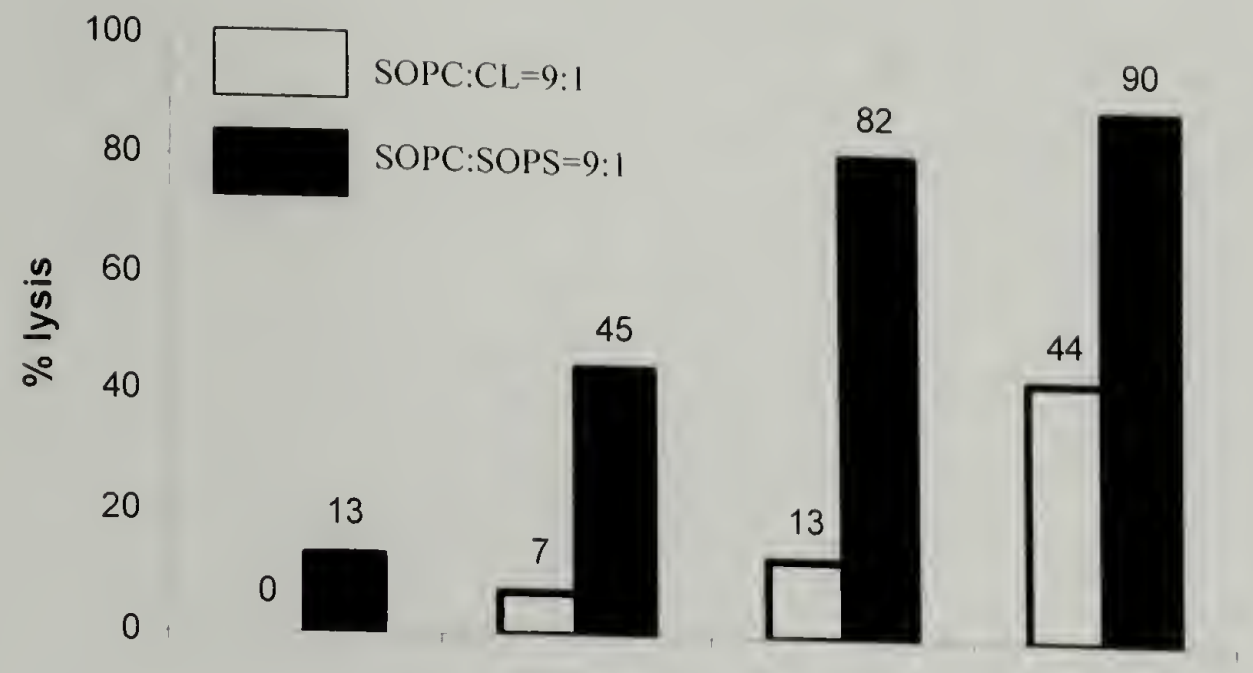


Figure 4.9 Lysis values 3 minutes after the addition of 25 μg/mL dep-poly**9** (M_n=9,950 g/mol, PDI=1.10), dep-poly(**9**$_2$-*co*-**11**$_1$) (M_n=15,300, PDI=1.15), dep-poly(**9**$_1$-*co*-**11**$_2$) (M_n=15,100, PDI=1.11) and dep-poly**11** (M_n=10,300, PDI=1.08) into suspensions of neutral (empty bars, SOPC: CL=9:1), anionic (full bars, SOPC: SOPS=9:1) liposomes.

It was observed that while the overall activities were decreased with decreasing content of **11** in the polymer, the selectivity was increased for both random copolymers. Both dep-poly(**9**$_2$-*co*-**11**$_1$) and dep-poly(**9**$_1$-*co*-**11**$_2$) have shown more than a six fold selective activity against anionic liposomes, as opposed to a near two fold selectivity of dep-poly**11**. Anionic liposomes respond to more hydrophobic **11** content of the polymer at smaller **11** contents than neutral liposomes resulting in the increased selectivity for random copolymers. The use of random copolymers allowed for the fine-tuning of the phospholipid membrane disruption activities of cationic amphiphilic polymers and achieved remarkable selectivities (Figure 4.10).

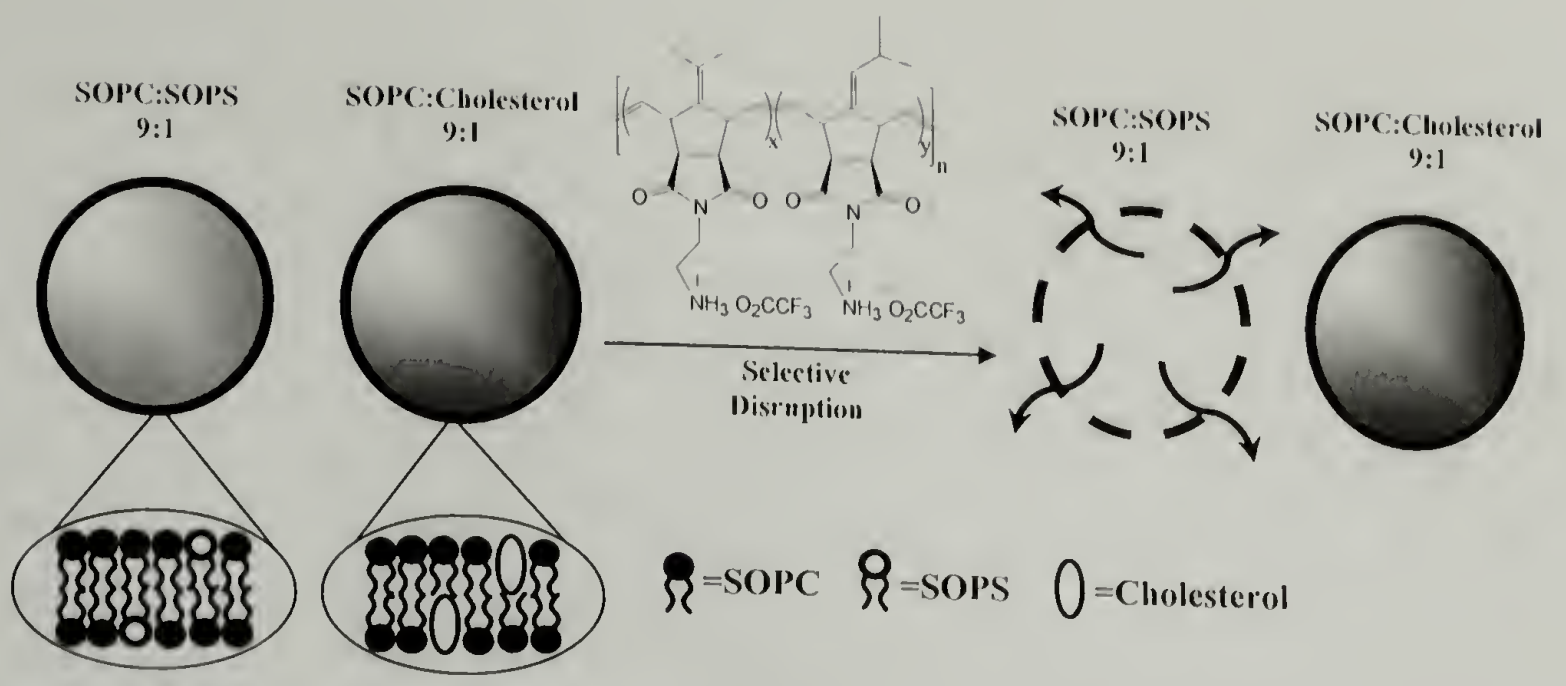


Figure 4.10 Illustration of selective membrane disruption activities of poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$).

4.3.6 Control Experiments

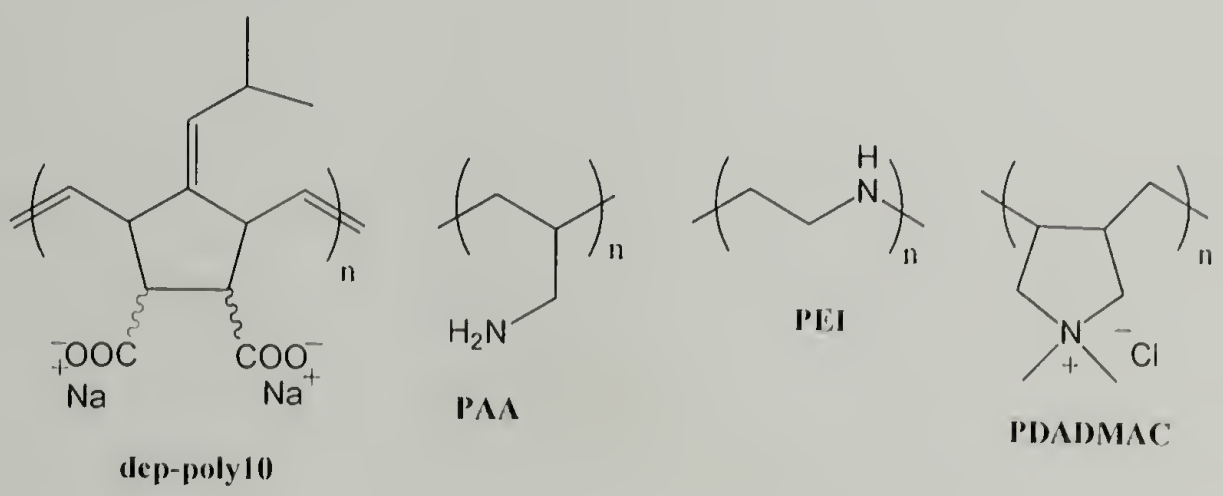


Figure 4.11 Structures of anionic analogue dep-poly**10**, polyallylamine (PAA), polyethyleneimine (PEI), and poly(diallyldimethyl ammonium chloride) (PDADMAC).

In a control experiment when the anionic dep-poly**10** (Figure 4.11, M_n = 22,000 g/mol) was added to the liposome suspensions no lysis was observed at comparable concentrations (Figure 4.12). It is remarkable that dep-poly**10**, which has the same hydrophobicity as the cationic dep-poly**11** that exhibited the highest membrane disruption activities against liposomes, did not cause lysis. A change from cationic to anionic character of the polymer resulted in a dramatic decrease of membrane disruption activity. Three commercially available cationic polymers, polyallylamine

(PAA, M_n = 25,000 g/mol), polyethyleneimine (PEI, M_n = 400,000 g/mol), and poly(dimethyldiallyl ammonium dichloride) (PDADMAC, M_n = 75,000 g/mol), were also tested as control experiments. These polymer samples provide models for primary amine (PAA), secondary and tertiary amine (hyperbranched PEI), and quaternary amine (PDADMAC) containing polymers, within a large range of high molecular weights. These polymers were observed to be far less active in the lysis of the lipid vesicles when compared to dep-poly**11** (Figure 4.12).

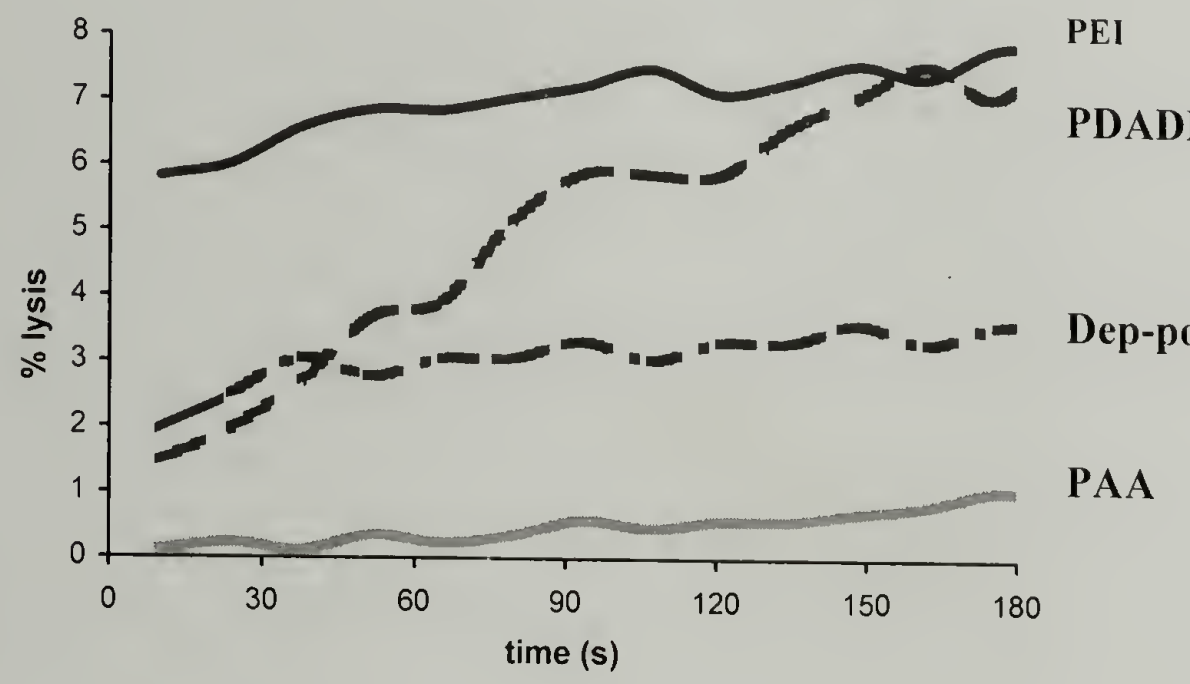


Figure 4.12 Lysis of anionic lipid vesicles caused by 15 μg/ml of dep-poly**10** (M_n = 22,000 g/mol), polyethyleneimine (PEI, M_n = 400,000 g/mol), poly(diallyldimethyl ammonium chloride) (PDADMAC, M_n = 75,000 g/mol), or poly(allylamine) (PAA, M_n = 25,000 g/mol).

These results once again confirmed that cationic amphiphilic polymer structures with a specific hydrophobicity have the highest activity for disruption of phospholipid membranes amongst the polymers studied.

4.4 Conclusion

Polymer induced fluorescent dye leakage from negatively charged and neutral large unilamellar vesicles (LUV) were measured. The amphiphilic polymers that were described in chapter 3 have been studied for their phospholipid membrane disruption activities. The level of control over the amphiphilic character on the repeating unit and molecular weight of polymers has been shown to play an important role in tuning the membrane disruption activities. The presence, and balance, of a hydrophobic group and a cationic group has been shown to be critical to achieve high activities. The membrane disruption activity of cationic amphiphilic polymers was found to reach a maximum at a critical molecular weight. These results suggested a cooperative action of these polymers in disrupting the phospholipid membranes. Lipid vesicles provide simplified models for bacterial and mammalian cell membranes although they underestimate several factors such as cell walls and lipopolysaccharides in bacterial cell membranes. However our results from membrane disruption activities of amphiphilic polymers built a strong foundation for structure property relationships of these materials and warrant further exploration of antibacterial activities as well as any other relevant biomedical application of these polymeric materials.

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CHAPTER 5

TUNING THE HEMOLYTIC AND ANTIBACTERIAL ACTIVITIES OF AMPHIPHILIC POLYNORBORNENE DERIVATIVES

5.1 Introduction

Antibacterial activities of macromolecules, including oligomeric compounds, have been studied under two major thrusts, for the most part independent from each other. One group of studies has focused on the structure-property relationships of natural host-defense peptides derived from multicellular organisms.[1-4] These peptides have a great diversity with regard to their length, amino acid composition and antimicrobial activities ranging from very potent to weak. Despite this diversity, most are cationic peptides with a certain degree of hydrophobicity. Extensive studies on the mechanism of action suggest that antimicrobial peptides act by permeabilizing the cell membranes of microorganisms through favorable interactions with negatively charged and hydrophobic components of the membranes followed by aggregation and subsequent disruption.[1,2,5,6] This mechanism is suggested to be responsible for the wide spectrum of potency and speed of action for these antibacterial peptides.[3] Host-defense peptides and their synthetic analogs are reported to exhibit varying degrees of activity against different bacteria and mammalian cells.[1] While host-defense peptides may show selectivity against the membranes of microbes versus the host organism, a number of them are antibacterial and not toxic to human cells, within certain concentration limits, and are thus considered as potential therapeutic agents.[1-3] Hemolytic activity against highly susceptible human red blood cells, as representatives of normal mammalian cells, is conventionally used as a measure of cytotoxicity.[4,5] The selective action has been suggested to be due to the balance and spatial arrangement of hydrophobic and

hydrophilic components of the peptide that distinguishes between the more negatively charged outer surface of microbial membranes and the neutral, cholesterol rich membranes of multicellular animals. Studies aimed at understanding the structure-property relationships of natural peptides have recently evolved into a number of research efforts targeting the preparation of synthetic mimics of antimicrobial peptides. These include stereoisomers of natural peptides,[7,8] α-peptides,[9] β-peptides,[10-13] cyclic α-peptides,[14] peptoids,[15] and polyarylamides,[16] all of which are oligomeric with molecular weight below 3,000 g/mol. Many of these examples target an amphiphilic secondary structure, typically helical, in addition to their cationic nature. Depending on the type of peptide, a facially amphiphilic structure results in the gain, or loss, of selective activity, which reveals that a stable amphiphilic secondary structure is not a precondition for selective antibacterial activity.[7,9,13] Resistance to enzymatic degradation was also targeted in some cases for potential use in therapeutic applications.[4,8,10,14,15]

Independent from the antimicrobial peptide research, a second thrust involves studies of synthetic cationic polymers that exhibit varying degrees of antibacterial activities.[17-23] This class of polymeric compounds is relatively inexpensive and less cumbersome to prepare when compared to peptide mimics. In many instances, cationic polymers were reported to exhibit enhanced antibacterial activities compared to their small molecule counterparts. The most common polymers are quaternary ammonium, or phosphonium functionalized. This class of cationic polymers was predominantly targeted for use in the solid state as potent disinfectants, biocidal coatings or filters, due to their toxicity to human cells at relatively low concentrations which is an important distinction from the work on peptide mimics.[17,18] Consistent with the targeted

applications of these cationic polymers, in most cases only antibacterial activity was reported without any report of hemolytic activity. In one instance, a soluble pyridinium polymer was reported to have low acute toxicity against the skin of test animals.[24] An example of antibacterial cationic polymers that have found large industrial use as disinfectants and biocides is poly(hexamethylene biguanide)s (PHMB) (Figure 5.1).

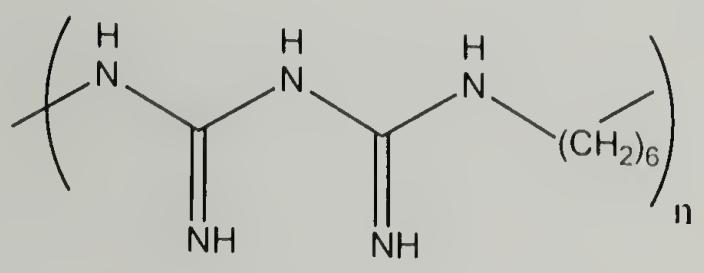

Figure 5.1 Poly(hexamethylene biguanide) (PHMB).

Different levels of toxicity against various mammalian cells were reported for PHMB and similar biguanide functionalized polymers.[25-29] To the best of our knowledge, a direct comparison of antibacterial and hemolytic action has not been reported for either of these classes of antimicrobial polymers. Gelman *et al.* has recently reported the antibacterial activity of low molecular weight, hydrophobically modified, cationic polystyrene derivatives in comparison with a potent derivative of magainin II.[30] In their initial study, a crossover between the research on antimicrobial peptide mimics and polymer disinfectants, cationic polystyrene derivatives has shown similar antibacterial activities as the magainin derivative, but were highly hemolytic. As a part of very recent efforts in the area, selective activities of facially amphiphilic low molecular weight polyphenyleneethynylenes were reported, with activity and selectivity similar to a magainin derivative.[31] The successful design of non-hemolytic, antibacterial, and high molecular weight polymers has not been achieved thus far.

Ring-opening metathesis polymerization (ROMP) has been successfully used in the preparation of biologically active well-defined polymeric materials,[32] due to its living nature and functional group tolerance.[33,34] Remarkable examples included polymers carrying oligopeptides,[35] oligonucleotides,[36] carbohydrates,[37-39] anti-cancer drugs,[40] and antibiotic agents.[41] ROMP-based techniques are evolving into a powerful synthetic toolbox for the introduction of multiple functionalities into polymeric materials in pursuit of obtaining potent biological activities. Chapter 3 of this thesis described the synthesis and ROMP of modular norbornene derivatives for the preparation of well-defined amphiphilic polymers exhibiting lipid membrane disruption activities.[42] In chapter 4 cationic amphiphilic polymers above a certain molecular weights were reported to show the highest membrane disruption activities on lipid vesicles as rough models for bacterial membranes.

This chapter presents the antibacterial and hemolytic activities of narrow polydispersity homopolymers and random copolymers of modular norbornene derivatives, spanning a large range of molecular weights. The results show that by controlling the hydrophobic/hydrophilic balance of water-soluble amphiphilic polymers, it is possible to obtain high selectivity between antibacterial and hemolytic activities without a predisposed amphiphilic secondary structure as part of the synthetic design. The overall efficacy toward both Gram-negative and Gram-positive bacteria is strongly dependent on the length of alkyl substituents on the repeat units. The results show that it is possible to design simple polymers that are both potent against bacteria, but non-hemolytic.

5.2 Experimental Section

5.2.1 Materials

All homopolymers and copolymers, dep-poly**9**, dep-poly**11**, dep-poly**12**, dep-poly**13**, dep-poly($\mathbf{9}_x$-*co*-$\mathbf{11}_y$)s, were prepared according to procedures described in Chapter 3. Reported molecular weights refer to values measured using THF GPC relative narrow molecular weight polystyrene standards, prior to the deprotection of polymers into their water-soluble cationic forms. All other reagents were obtained from Aldrich.

5.2.2 Instrumentation

Optical density and absorbance spectroscopy were recorded with a Molecular Devices SpectraMAX 190 plate reader.

5.2.3 Measurement of Hemolytic Activity

Hemolytic activity measurements were performed with a slight modification of literature procedures.[7,12,43] Freshly drawn human red blood cells (HRBC, 30μL), were suspended in 10 mL TRIS saline (10 mM TRIS, 150 mM NaCl, pH 7.2, filtered through polyethersulfone membrane with 0.20 μm pore size) and rinsed 3 times by centrifugation (5 minutes at 1500 rpm) and resuspended in TRIS saline. Polymer solutions were prepared by dissolution in TRIS saline (10 mM TRIS, 150 mM NaCl, pH 7.2) at concentration of 8 mg/mL and further diluted as necessary. After the complete dissolution the pH of the solution was adjusted to values between 6.5 and 7.0 depending on the solubility of polymer. TRIS saline solutions of dep-poly**9**, dep-poly**13**, and dep-poly(**9**-*co*-**11**) were adjusted to pH 7.0. TRIS saline solutions of dep-poly**11**, and dep-poly**12** were adjusted to pH 6.5 because of slow precipitation of these polymers at higher pH values. After the pH adjustments, polymer solutions were filtered through

polyethersulfone membranes (0.45 μm pore size). Freshly prepared polymer solutions with different concentrations were added to 100 μL of the above-prepared HRBC suspension to reach a final volume of 200 μL on a 96-well plate. The resulting mixture was kept at 37˚C for 30 minutes on a stirring plate. Then the plate was centrifuged (IEC Centra-4B, 10 minutes at 1500 rpm) and the supernatant in each well was transferred to a new plate. Hemolysis was monitored by measuring the absorbance of the released hemoglobin at 414 nm. 100% hemolysis was obtained by adding 10 μL of TRITON-X solution (20% by volume in DMSO), a strong surfactant, to the above-prepared HRBC suspension. The upper limit of polymer concentration that was required to cause 50% hemolysis is reported as HC_{50}, where the absorbance from TRIS saline containing no polymer was used as 0% hemolysis. The value of percent hemolysis was reported in cases where it was below 50% hemolysis at the highest polymer concentration tested or above 50% hemolysis at the lowest polymer concentration tested. Relatively small absorbance of polymer solution due to residual catalyst at 414 nm, at the corresponding concentrations, were measured and subtracted from polymer-HRBC mixtures. All experiments were run in quadruplicate. Control experiments were run in order to monitor the hemolytic activity of TFA treated ruthenium catalyst that may be present in trace amounts in polymer solutions. Catalyst was dissolved and stirred for 8 hours at 45˚C in TFA followed by evaporation of TFA and dissolution in DMSO due to the insolubility of TFA treated catalyst in TRIS saline. It must be noted that as a result of successive precipitations of the protected polymer in pentane, the majority of the initial ruthenium catalyst was removed from the polymer. Elemental analysis, which was performed to determine the residual ruthenium in the deprotected polymer, did not show

the presence of ruthenium down to 0.3 wt% for dep-poly**9** (M_n=9,950 g/mol), and dep-poly**11** (M_n=10,050 g/mol). No hemolytic activity was observed from the TFA treated catalyst up to a concentration of 100 μg/mL (10 μL of 2 mg/mL solution in DMSO), a higher concentration than that possible due to residual catalyst at the highest polymer coneentrations.

5.2.4 Measurement of Antibacterial Activity

Antibaeterial aetivity measurements were performed with slight modifications of literature proecdures.[7,12,31] Bacteria suspension (*E. coli* D31 and *B. subtilis* ATCC 8037), whieh was grown in Mueller-Hinton Broth (MHB) overnight at 37°C, diluted with fresh MHB to an optical density of 0.1 at 600 nm (OD_{600}) and further diluted by a factor of 10. This suspension was mixed with different eoncentrations of freshly prepared polymer solutions in TRIS saline (pH 6.5-7.0), by serial dilutions in a 96-well plate and incubated for 6 hours at 37°C. The OD_{600} was measured for bacteria suspensions that were ineubatcd in the presenee of polymer solution or only TRIS saline. Antibaeterial aetivity was expressed as minimal inhibitory eonecntration (MIC), the concentration at whieh 90% inhibition of growth was observed after 8 hours. All experiments were run in quadruplicate. In a control experiment, the TFA treated ruthenium eatalyst did not show any antibaeterial activity within the time and eoneentration limits that were used for antibaeterial activity assays.

5.3 Results and Discussion

5.3.1 Amphiphilic Polynorbornene Derivatives

We probed the biologieal aetivities of a class of amphiphilie polymers that were previously shown to cxhibit lipid membrane disruption aetivities (Chapter 4).[42] The amphiphilic polynorbornene derivatives bearing primary amine and variable length

alkyl moieties as pendant groups were prepared by ROMP of modular norbornene derivatives using catalyst **4**, the Grubbs-Love catalyst.[44] These amphiphilic polymers provide a well-defined model for testing the effect of hydrophobicity and molecular weight of cationic polymers on antibacterial and hemolytic activities. The current study involves four types of repeating units, **9**, **11**, **12**, and **13**, as shown in Figure 5.2. All homo and copolymers of these monomers have narrow polydispersities, less than 1.3, and encompass a large range of molecular weight from oligomers to high polymers, up to 137,500 g/mol, as determined by THF GPC relative to polystyrene standards prior to the deprotection of polymer. No preformed and stable polymeric secondary structure is expected from these macromolecules considering the imperfect tacticity of polynorbornene derivatives prepared by homogeneous ruthenium catalyst,[34,45] and the presence of *cis-trans* isomers on the backbone unsaturations. Furthermore, the asymmetry in the isobutylidene group of dep-poly**11** results in head-to-head and head-to-tail insertions leads to multiple dyad possibilities. In the case of random copolymers there is the added factor of compositional heterogeneity. All deprotected polymers are soluble in TRIS saline solutions at appropriate pH values (6.5-7.0).

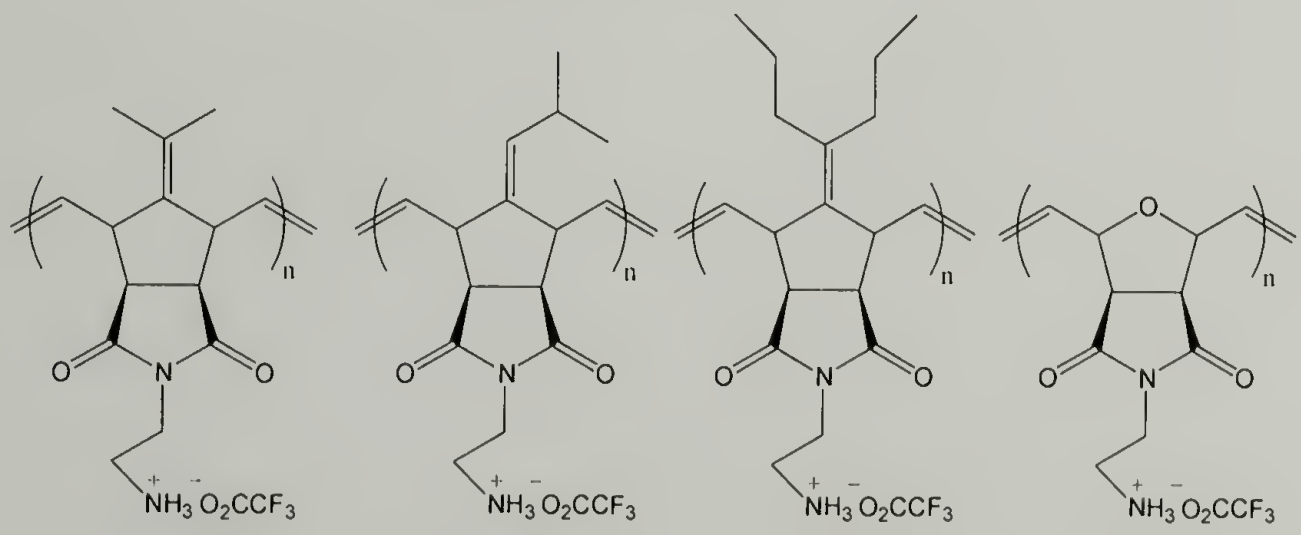

Figure 5.2 Amphiphilic polynorbornene derivatives.

5.3.2 Antibacterial and Hemolytic Activities of Homopolymers

The hydrophobicity of the repeating unit was observed to have dramatic effect on antibacterial and hemolytic activities of the amphiphilic polymers. The activity of each homopolymers with similar molecular weights (near 10,000 g/mol, M_n) was probed against Gram-negative bacteria (*E. coli*), Gram-positive bacteria (*B. subtilis)*, and human red blood cells (Table 5.1).

Table 5.1 Antibacterial and hemolytic activities of homopolymers

Polymer	M_n (g/mol)	PDI	MIC [μg/mL, (μM)]		HC_{50} [μg/mL, (μM)]	Selectivity (HC_{50}/MIC)	
			E. coli	*B. subtilis*		*E. coli*	*B. Subtilis*
Dep-Poly**13**	10,250	1.07	>500, (>49)	>500, (>49)	>1000, (>98)	-	-
Dep-Poly**9**	9,950	1.10	200, (20)	300, (30)	>4000, (>400)	>20	>13
Dep-Poly**11**	10,050	1.13	25, (2.5)	25, (2.5)	<1, (<0.1)	<0.04	<0.04
Dep-Poly**12**	10,300	1.08	200, (19)	200, (19)	<1, (<0.1)	<0.005	<0.005

Dep-poly**13**, a cationic polymer with no substantial hydrophobic group, did not show any significant antibacterial or hemolytic activity within the measured concentrations. At the highest concentration measured for hemolytic activity, 1000 μg/mL, dep-poly**13** caused 5% hemolysis. This result is consistent with the lack of activity against phospholipid membranes reported in Chapter 4. Introduction of a hydrophobic group at the repeat unit level produced an increase in antibacterial and hemolytic activities, which depended on the size of hydrophobic group. Dep-poly**9**, with an isopropylidene pendant group, exhibited antibacterial activity with MIC of 200 μg/mL against *E. coli*, which is less efficacious than most antimicrobial peptides, and their mimic, that have MICs typically ranging between 1-50 μg/mL.[1,3,8,9,11-16,46]

However the hemolytic activity of dep-poly**9** remained below 5% up to 3000 μg/mL (Figure 5.3), a value well above its MIC. Above 3000 μg/mL the hemolytic activity of this polymer increase more rapidly with increasing concentration. Increase in hemolysis, to 25%, at 4000 μg/mL could be induced through different mechanisms, such as increased osmotic pressure at high polymer concentration, rather than a local membrane perturbation. However HC_{50} value remained above the measured concentration of 4000 μg/mL, thus giving a selectivity, defined as the ratio of HC_{50} to MIC,[16] greater than 20.

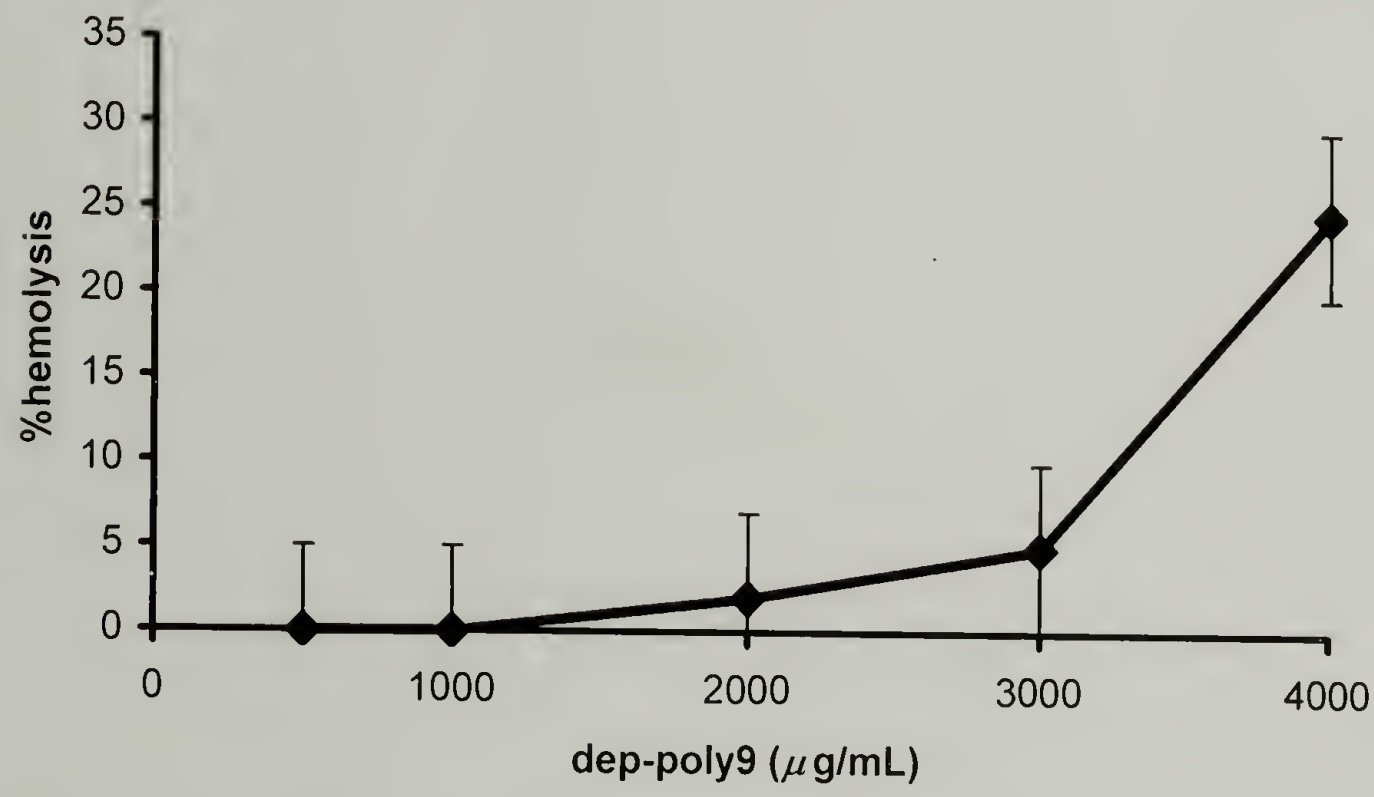

Figure 5.3 Concentration dependent hemolysis caused by dep-poly**9**, 9,950 g/mol (M_n).

Dep-poly**11** with an additional carbon atom per repeat unit is more hydrophobic than dep-poly**9**, and has additional mobility of the pendant alkyl group. Dep-poly**11** exhibited substantial increase in antibacterial activity, with MIC of 25μg/mL for both *E. coli* and *B. subtilis* as well as hemolytic activity, HC_{50} less than 1 μg/mL, with an 80% hemolysis at 1 μg/mL (Table 5.1). This increase in antibacterial and hemolytic activity

with increasing hydrophobicity is in accordance with literature reports that predict larger hydrophobic groups will have stronger interactions with the inner core of cell membranes leading to loss of selectivity.[1-4] In the case of dep-poly**12**, when the hydrophobic size was further increased the hemolytic activity was retained with a 100% hemolysis at 1 μg/mL, however the antibacterial activity decreased to a MIC of 200 μg/mL. In many instances, hydrophobic interactions have been reported to control hemolytic activities; whereas charge interactions are suggested to be more important for antibacterial activity.[1,9] These results show that the presence, and balance, of hydrophobic and hydrophilic groups dictate the antibacterial and hemolytic activities of the amphiphilic non-natural polymer in agreement with natural peptide studies.

The effect of molecular weight on antibacterial and hemolytic activities was investigated for dep-poly**9**, dep-poly**11**, and dep-poly**12** (Table 5.2). Changes in molecular weights over a large range did not result in significant changes in antibacterial and hemolytic activities of dep-poly**9** and dep-poly**12**. The antibacterial activity of dep-poly**11** was observed to increase moderately as the molecular weight decreased from 57,200 g/mol to 10,300 g/mol or lower. Overall there was no substantial molecular weight dependence on antibacterial or hemolytic activities of these homopolymers if activity is reported in mass/volume rather than molarity. In the most commonly suggested mechanisms for membrane disruption based on amphiphilic peptides, there is some type of cooperative action, either in pore formation or coverage of the surface in a carpet-like manner.[2,5] If the membrane disruption activity is associated with the accumulation of the macromolecule on the membrane surface, it is a germane approach to report MIC values in units of mass/volume. Otherwise at the same

molar concentrations higher molecular weight polymers would cover larger surfaces than lower molecular weight polymers. However, it should be noted that this approach underestimates the possible effect of the increase in the number of electrostatic and hydrophobic interactions at the membrane surface as a consequence of covalent connectivity resulting from higher molecular weights. One of many possible advantages of high molecular weight polymeric systems would be the ability of using them at relatively low molar concentrations if that is a requirement of the target application.

Table 5.2 Effect of molecular weight on antibacterial and hemolytic activities

Polymer	M_n (g/mol)	PDI	MIC [μg/mL, (μM)]		HC_{50} [μg/mL, (μM)]
			E. coli	*B. subtilis*	
Dep-Poly**9**	1,600	1.15	200, (125)	300, (188)	>4000, (>2500)
	24,100	1.10	200, (8.3)	200, (8.3)	>4000, (>164)
	49,600	1.14	200, (4.0)	200, (4.0)	>4000, (>81)
	137,500	1.27	200, (1.5)	200, (1.5)	>4000, (>29)
Dep-Poly**11**	1,650	1.26	25, (15)	25, (15)	<1, (<0.6)
	25,500	1.17	40, (1.6)	40, (1.6)	<1, (<0.04)
	57,200	1.70	80, (1.4)	80, (1.4)	<1, (<0.02)
Dep-Poly**12**	5,300	1.09	200, (38)	200, (38)	<1, (<0.2)
	32,200	1.13	200, (6.2)	200, (6.2)	<1, (<0.04)
	57,000	1.19	200, (3.5)	200, (3.5)	<1, (<0.02)

Dep-poly**9**s caused 20-25% hemolysis at 4000 μg/mL. Dep-poly**11**s caused 70-80% hemolysis at 1 μg/mL. Dep-poly**12**s caused 100% hemolysis at 1 μg/mL.

5.3.3 Antibacterial and Hemolytic Activities of Random Copolymers

The results from homopolymerization studies have shown the strong influence of subtle structural changes on the biological activities of these amphiphilic polymers.

The low hemolytic activity of dep-poly**9** and strong antibacterial activity of dep-poly**11** suggests that copolymerization of monomers **9** and **11** would be a facile synthetic approach to optimize activity and selectivity. The preparation and characterization of random copolymers consisting of different comonomer ratios of **2** and **3** were described in Chapter 3. Our synthetic approach was shown to allow for various compositions to be readily explored. Dep-poly($\mathbf{9}_9$-*co*-$\mathbf{11}_1$), the random copolymer of **9** and **11** with a final comonomer molar ratio of 9/1 respectively and M_n of 12,000 g/mol, showed antibacterial activity near that of dep-poly**11** while retaining the non-hemolytic character of dep-poly**9** (Table 5.3). Remarkably, 10 mol% of comonomer **11** was enough to bring the antibacterial activity near that of homopolymer dep-poly**11** and still exhibit excellent selectivity ratios greater than 100. Dep-poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$)s, of two different molecular weights, have also shown high selectivity where antibacterial activity was slightly decreased with increasing molecular weight as in the case of dep-poly**11**.

Table 5.3 Activities of random copolymers of **9** and **11**

Polymer	**M_n (g/mol)**	**PDI**	**MIC [μg/mL, (μM)]**		**HC_{50} [μg/mL, (μM)]**	**Selectivity (HC_{50}/MIC)**	
			E. coli	***B. subtilis***		***E. Coli***	***B. subtilis***
Dep-Poly($\mathbf{9}_9$-*co*-$\mathbf{11}_1$)	12,000	1.09	40, (3.3)	40, (3.3)	>4000, (>333)	>100	>100
Dep-Poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$)	15,300	1.15	40, (2.6)	40, (2.6)	>4000, (>261)	>100	>100
	93,700	1.21	80, (0.9)	80, (0.9)	>4000, (>43)	>50	>50
Dep-Poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$)	8,500	1.09	40, (4.7)	40, (4.7)	<1, (<0.12)	<0.025	<0.025
	32,600	1.19	80, (2.5)	80, (2.5)	<1, (<0.03)	<0.013	<0.013
Dep-Poly($\mathbf{9}_1$-*co*-$\mathbf{11}_4$)	11,800	1.15	40, (3.4)	40, (3.4)	<1, (0.08)	<0.025	<0.025

Table 5.4 Percent hemolysis values at the lower and upper limits of HC_{50} measurements for homopolymers and random copolymers

Polymer	M_n (g/mol)	PDI	%Hemolysis at	
			4000 μg/mL	1 μg/mL
Dep-Poly**9**	1,600	1.15	24	-
	137,500	1.27	22	
Dep-Poly($\mathbf{9}_9$-*co*-$\mathbf{11}_1$)	12,000	1.09	15	-
Dep-Poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$)	15,300	1.15	25	-
	93,700	1.21	20	-
Dep-Poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$)	8,500	1.09	-	68
	32,600	1.19	-	60
Dep-Poly($\mathbf{9}_1$-*co*-$\mathbf{11}_4$)	11,800	1.15	-	75
Dep-Poly**11**	1,650	1.26	-	78
	57,200	1.70	-	70
Dep-Poly**12**	5,300	1.09	-	100
	57,000	1.19	-	100

Similar to dep-poly**9**s, dep-poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$) of 15,300 g/mol (M_n), caused less than 5% hemolysis up to a concentration of 2000 μg/mL, HC_{50} remaining above 4000 μg/mL (Figure 5.4). It is remarkable that there is a 50-fold difference between the MIC, and the concentration at which there is almost no hemolytic activity. These copolymers, with selectivity values reaching over 100, are powerful examples of the ability to obtain antibacterial activity from non-hemolytic polymers by fine-tuning the hydrophobic/hydrophilic balance and molecular weight.

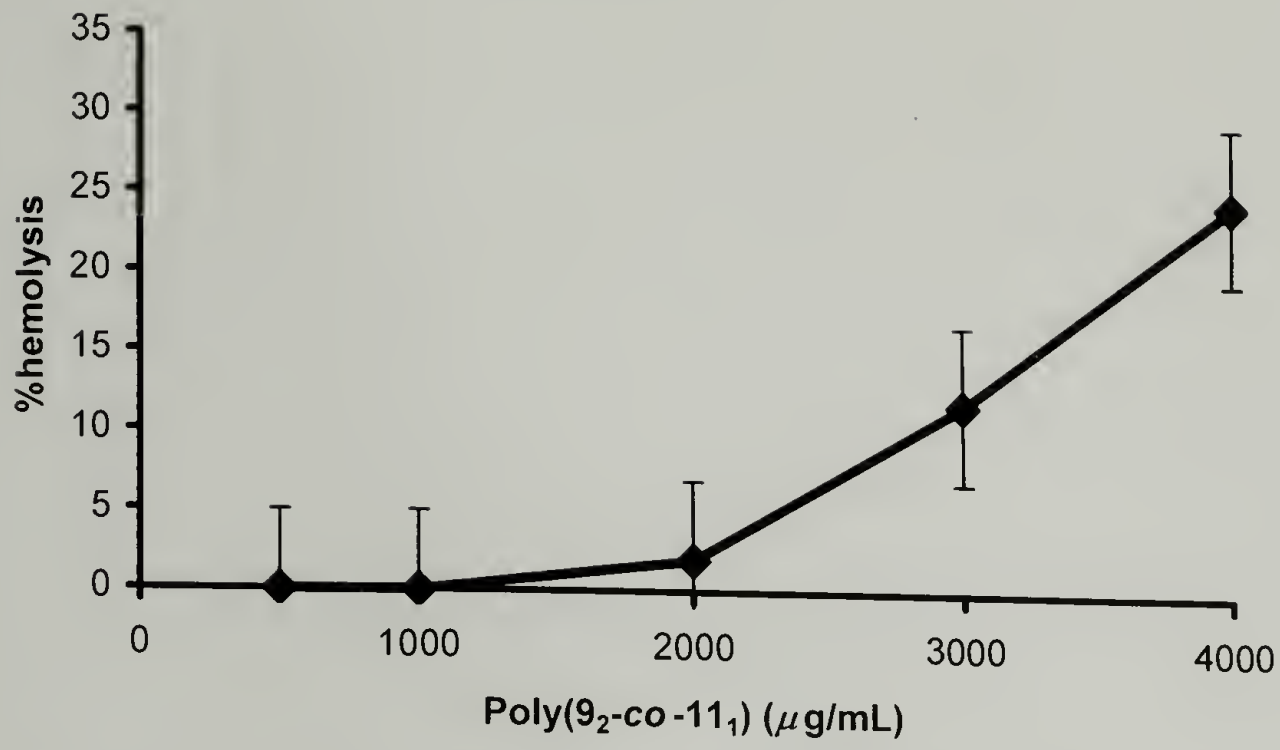

Figure 5.4 Concentration dependent hemolysis caused by dep-poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$), 15,300 g/mol (M_n).

It was previously suggested in the literature that a comparison between new compounds and a reference peptide would be the best indicator for clinical cytotoxicity, and allow a better comparison between different antibacterial agents from different laboratories.[43] In a control experiment, the activity of a Magainin derivative (MSI-78), a well-known antimicrobial peptide, was measured against the same *E. coli* strain. In comparison to above described homopolymers and copolymers MSI-78 exhibited a selectivity of 9.6 that was calculated from an MIC of 12.5 μg/mL and HC_{50} of 120 μg/mL (Table 5.5).

Table 5.5 Comparisons of selective activities

Polymer	**MIC (*E. coli*) [μg/mL]**	**HC_{50} [μg/mL]**	**Selectivity (HC_{50}/MIC)**
Dep-Poly**9**	200	>4000	>20
Dep-Poly($\mathbf{9}_9$-*co*-$\mathbf{11}_1$)	40	>4000	>100
Dep-Poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$)	40	>4000	>100
MSI-78	12.5	120	9.6

Random copolymers dep-poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$)s and dep-poly($\mathbf{9}_1$-*co*-$\mathbf{11}_4$)s exhibited high hemolytic activities in accordance with the increased content of hemolytic comonomer **11**. In these copolymers the selective activity against bacteria was lost and reversed into a selective activity against HRBC, exhibited at concentrations lower than 40 μg/mL.

5.3.4 Experimental Considerations: Effect of Blood Freshness

All hemolysis results that are reported in this thesis were obtained using freshly drawn blood from one individual. During the course of this study, the hemolytic activities of dep-poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$)s were observed to be dependent on the freshness of the blood. Differences for this polymer were also noted for blood obtained from different individuals. It was determined that blood that was stored for more than 7 days was more susceptible to hemolysis than freshly drawn blood. The HC_{50} of dep-poly($\mathbf{9}_1$-*co*-$\mathbf{11}_2$)s that was below 1 μg/mL against freshly drawn blood from one individual, was observed to be above 4000 μg/mL against freshly drawn blood from another individual. These observations were in accordance with previous literature that reported higher susceptibility to hemolysis, caused by a series of cationic antimicrobial peptides, in the case of blood stored for 21 days in 4°C as opposed to fresh blood.[43] Blood susceptibility to hemolysis was also reported to depend on the difference in ionic strength of the test medium, and the blood drawn from different individuals. It was suggested that as the blood gets old, the loss of natural restoration mechanisms against disruptions in cellular membranes, such as protein pumps that remove foreign objects from cell surfaces, could result in increased susceptibility. Non-hemolytic homopolymers and copolymers, dep-poly**9**, dep-poly**13**, dep-poly($\mathbf{9}_9$-*co*-$\mathbf{11}_1$), and dep-poly($\mathbf{9}_2$-*co*-$\mathbf{11}_1$), remained non-

hemolytic against old blood that was stored for 3 weeks at 4°C, and blood from different individuals, with HC_{50} values above 4000 μg/mL.

5.3.5 Applications for Well-Defined Amphiphilic Polynorbornene Derivatives

5.3.5.1 Advantages of ROMP-Based Synthetic Strategy

As mentioned in Chapter 1, amphiphilic polymers have attracted attention for a number of biomedical and therapeutic application. A variety of amphiphilic polymer architecture was considered either as delivery agents for drugs[47-49] and genes,[50-52] structural components in tissue engineering,[53,54] or active therapeutics, such as antibacterial agents.[4,19-21] Several attributes of amphiphilic polynorbornene derivatives, based on modular norbornene derivatives, raise their potentials to be used for many of these applications. The high level of control over amphiphilic character and molecular weights of the polymer is desirable for all applications as it provides flexibility to fine-tune material properties and fulfill necessary safety requirements. For example, the access to water-soluble polymers and control over molecular weights are important factors for many biomedical applications in terms of better transport in biological environments. A number of reports described the use of ROMP for the preparation of polymers decorated with biologically active agents, including peptides,[35] carbohydrates,[37] oligonucleotides,[36] antibiotic agents,[41] and anti-cancer drugs.[40] These unique materials with high local density of the active groups in the vicinity of the polymer chains warrant further evaluations in therapeutic applications. However these materials are commonly associated with a number of limitations such as poor solubility, or inefficient transport through biological membranes. These synthetic approaches can easily be combined with our approach through copolymerizations, in order to incorporate various polymer segments with distinct, and complementary biological

activities. A proper choice of membrane disrupting amphiphilic block, based on modular norbornene derivatives, could provide selective antibacterial activity, as well as facilitate delivery of these multi-component polymeric agents through mammalian cell membranes. With a powerful set of ROMP-based synthetic approaches available, and careful monomer design, the potency of polymeric therapeutic agents can thus be fine-tuned.

5.3.5.2 Applications for Polymeric Non-toxic Antibacterial Agents

While well-defined amphiphilic polynorbornene derivatives possess properties desirable for a number of biomedical applications, this chapter has focused on the antibacterial activities of amphiphilic polymers in aqueous solutions. The growing problem of multi-drug-resistant bacteria stimulated the development and evaluation of new antibacterial agents.[2,55] Antimicrobial peptides and their synthetic mimics have attracted great attention in this respect.[1,2,4]

There have been several considerations about the potency and safe use of such peptidic and polymeric agents.[56] The resistance to enzymatic degradation is one concern related to the potency of a drug. The all hydrocarbon backbone of polynorbornene derivatives provides a stable chain structure in biological media. However, the unsaturations in the backbone, despite the surrounding steric constraints, could face the problem of oxidation in the long term. Late transition metal catalyzed, or hydrazine mediated hydrogenation of backbone unsaturations, which has been extensively used for functionalized ROMP polymers, could provide a facile solution to the potential oxidation problem.[57-64] Although the biological activities of amphiphilic polymers could be altered by increased flexibility on the hydrogenated polymer backbone and

hydrophobic side groups, the principles that were established to tune the hemolytic and antibacterial activities of the parent polynorbornene derivatives would still be pertinent.

A second point of concern is the five membered imide ring of the amphiphilic polynorbornene derivatives which could be a potential site for enzymatic reactions. The ring-opening hydrolysis reaction of the cyclic imide was suggested to be a key intermediate in the degradation of proteins and peptides.[65] Many types of cyclic imides are known to be hydrolyzed by the mammalian enzyme dihydropyrimidinase.[66,67] Similarly, a more substrate specific enzyme, imidase, was also found in bacteria.[66] A thorough study of the enzymatic degradation of imide side chains may be necessary. However, when the substrate specificity of these enzymes is considered, the *N*-2-ethylamine substituent on the imide side group of amphiphilic polynorbornene derivatives, and the abiogenic polymeric structure are expected to provide stability against enzymatic degradation. In addition, the access to a large range of molecular weights without compromising the selective antibacterial activities could be advantageous to tune enzymatic stability.

Another crucial factor for therapeutic applications of antimicrobial peptides, either oligomeric or polymeric agents is the toxicity, and immunogenicity[2-4,68] of these compounds along with their distribution and excretion from the body.[4,24,68-70] For all therapeutic applications a thorough investigation of these properties is required. The ability to obtain a very low hemolytic activity ($HC_{50} > 4$ g/mL), against highly susceptible human red blood cell, while retaining good antibacterial activities (MIC (*E. coli)* = 40 μg/mL) suggests amphiphilic polynorbornene derivatives to be considered for more detailed toxicity and immunogenicity tests. A non-ionic polymer, poly(ethylene

oxide-*b*-propylene oxide), was reported to be clinically well tolerated in a number of mammalian species for its distribution, metabolism, and excretion.[69] The distribution and excretion of polymeric materials from the body was suggested to be partly dictated by its molecular weight. Once again the ability to obtain desired biological activities over a large range of molecular weights raise the potential of amphiphilic polynorbornene derivatives to be evaluated for their distribution in, and excretion from, mammals.

Because of the above-mentioned safety concerns polymeric antibacterial agents were mostly targeted for use in topical as opposed to systemic applications.[2] This is largely due to the relative safety of topical therapy. Examples of topical therapy include anti-infective wound healing agents and antifungal agents. An important consideration is the cost aspects of such topical antibacterial agents. Synthetic peptides were reported to be several fold more expensive than conventional antibiotics.[4] While new synthetic strategies and recombinant techniques are widely screened for decreasing the cost of peptide based antimicrobial agents, our work, along with a limited number of other research efforts,[24,30,31,71] show the potential of using new abiogenic polymer structures as antibacterial agents.

A number of literature reports demonstrated the potential of cationic antimicrobial peptide mimics as antitumor agents in cancer therapy[1,3,72-74] and antiviral agents,[1,3] where the mechanisms for antitumor and antiviral activities are under investigation. Thus cost effective, non-hemolytic antibacterial polynorbornene derivatives with controlled structures can also be considered for their anti-cancer and antiviral activities. Tumor cells were shown to be more susceptible to cationic

antimicrobial peptides than healthy cells, due to a number of possible reasons including the cell surface exposure of negatively charged phosphatidylserine, changes in membrane potential due to higher metabolism, and alterations in extracellular matrix in cancer cells. Antiviral activity was suggested to be through inhibitory absorption of peptide or polymer on the viral particles or by perturbing the protein assembly of the virus.[1,75]

Finally, as described in the introduction section of this chapter, a large number of cationic polymers have been developed as biocidal agents.[18] Due to their toxicity profiles cationic biocidal polymers were suggested for use in the solid state, such as active agents for water and air purification filters, coatings against biofouling, additives for textile fibers, or preservatives in paints, waxes, and oils. Certain materials properties are required for such applications, such as long-term stability and activity, no leaching or decomposition into toxic products, along with cost efficiency. Although amphiphilic polynorbornene derivatives described in this dissertation were studied for their solution activities their use in the solid state could be desirable for various reasons. Although the cost efficiency of amphiphilic polynorbornene derivatives compared to other inexpensive polymeric biocides can be debated there are several strong assets associated with our system. The well-defined character of ROMP allows for this type of polymers to be chemically anchored into various substrates.[76-78] In addition highly nucleophilic primary amine functionality on the side groups also allow for several chemical approaches to be considered for surface attachment through nucleophilic attack. The control over the hydrophobic/hydrophilic balance can also be advantageous for tuning the incorporation and compatibility of these polymers in other polymeric systems that

are used as coatings or textile fibers. Very low hemolytic activity of specific polynorbornene derivatives is also desirable in applications where leaching from surface is a high possibility.

5.4 Conclusions

The motivation in Chapter 3 was to develop amenable synthetic approaches for the preparation of amphiphilic polymers with well-controlled structures that would broaden the interface between macromolecular science and biological sciences. Following those efforts, in this chapter amphiphilic polymers based on modular norbornene derivatives were shown to exhibit good antibacterial activities and high selectivity for bacteria versus red blood cells. Small modifications to the hydrophobic character of the cationic amphiphilic polymer were shown to dramatically change the antibacterial and hemolytic activities. Tuning the hydrophilic/hydrophobic balance and molecular weights of these copolymers allowed preparation of highly selective, antibacterial non-hemolytic macromolecules. Desired biological activities were maintained across a large range of molecular weights. Furthermore, this study showed the preparation of fully synthetic high molecular weight polymers that mimic the activities of host-defense peptides in the absence of a specific secondary structure.

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