2014

# Polyethylene Glycol containing ROMPolymers for the Modification of Neuro-Protective Hemoglobin

Andrea L. McCollum

**Polyethylene Glycol containing ROMPolymers for the Modification of Neuro-Protective Hemoglobin**

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Chemistry

By

Andrea Lindsey McCollum

Under the mentorship of Dr. Hans-Joerg Schanz

ABSTRACT

Polyethylene glycol (PEG) has shown the ability to improve compatibility when used in combination with cell-free hemoglobin in the treatment of traumatic brain injuries. It has been demonstrated that the covalently bonded PEG increases the hydrodynamic radius of the hemoglobin and hence generates a physical barrier while slowing down the oxygen delivery of the strongly oxidative hemoglobin. In this context, I have been working on the development of synthetic pathways to incorporate PEG into monomers and polymers through both direct modification of (7-oxa)norbornene derivatives and post polymerization modification. Starting from the (7-oxa)norbornene anhydride derivatives, we have developed pathways to cationic and uncharged monomers which can be polymerized via Ring Opening Metathesis Polymerization (ROMP) using Grubbs 1$^{st}$ and 3$^{rd}$ generation-type Ru-alkylidene complexes. These complexes are known to produce ROMPolymers with high molecular weight control. In this context, we also developed a chain transfer agent to introduce a functional end group into the polymer which will enable the covalent binding of the polymer to the hemoglobin protein.

Thesis Mentor:_____

Dr. Hans-Joerg Schanz

Honors Director:_____

Dr. Steven Engel

April 2013
Chemistry
University Honors Program
**Georgia Southern University**

# INTRODUCTION

A life essential protein of the human body is found in red blood cells, the hemoglobin. The hemoglobin primarily functions to collect oxygen from the lungs and distribute it to other tissues throughout the body. The hemoglobin protein is unique in that it can be isolated from red blood cells and not lose functionality; the protein maintains its full oxygen binding capacities and properties making it an object of focus as a therapeutic oxygen delivery system. The isolated hemoglobin protein is dubbed cell-free hemoglobin or free hemoglobin. Over the past decade, much time and research efforts have been dedicated towards the development of using cell-free hemoglobin for medicinal purposes. As of now, three cell-free hemoglobin based oxygen carrier (HBOC) products have been used in human trials for the treatment of traumatic brain injuries (TBI). Unfortunately, these HBOC products have not been approved by the FDA due to the side effects.

Every nine seconds, a human being sustains a traumatic brain injury (TBI). TBI are serious and widespread. The mortality rate associated with TBI increases when complicated by a secondary issues i.e. hemorrhagic shock, encephalitis, hypotension (low blood pressure). Hypotension results in less blood and oxygen flowing to the tissues throughout the body. Patients with TBI are extremely susceptible to hypotension; damage to the neural network is due to the lack of proper oxygen circulation to effected areas. This establishes a crucial need for modified HBOC products as part of a treatment which restores blood pressure and oxygen delivery to the site of the TBI.

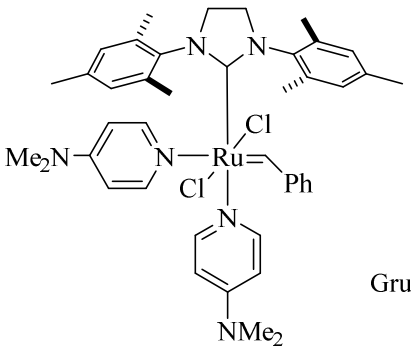
As previously mentioned, no HBOC product has been approved by the FDA for the treatment of traumatic brain injuries. The problem with current HBOC products is the

toxicity of reactive oxygen species (ROS) produced when the HBOC is introduced to the body. In this context, HBOC products bind oxygen as effectively as red blood cells; however, when the hemoglobin is removed from the cell, it loses its ability to detoxify the superoxide radical species. The new generation of HBOC products will, in theory, be able to bind oxygen while quenching the toxic radical as a result of premature oxygen release. A possible candidate for this is polynitroxyl pegylated hemoglobin (PNPH).[1-6] PNPH has demonstrated a lower toxicity for *in vitro* and *in vivo* models. These covalently attached modifications contain an antioxidant to prevent ROS formation. After the antioxidant is added to the hemoglobin, multiple polyethylene glycol chains are tethered to the hemoglobin in order to increase the size and half-life of the HBOC. The current synthetic methods are extensive, not very efficient and expensive; improving the current procedure should improve the practicality and marketability of using HBOC products to reverse TBI.[7-8]

In polymer synthesis, a powerful tool has been growing in popularity over the past few decades: ring opening metathesis polymerization (ROMP). [9-11] ROMP can be conducted at moderate temperatures and the Ru-alkylidene complexes are compatible with many sensitive functional groups. ROMP is an ideal technique for the synthesis of (co)polymers with sensitive biological functional groups as present in our targeted materials. Another ideal feature of ROMP is that the polymerization proceeds continuously until addition of a terminating agent; hence, the polymerization is "living". This aspect allows both the molecular weight and physical properties of polymers to be regulated with a high amount of precision with the use of an appropriate catalyst. The combination of these features makes ROMP the most suitable technique for the project.

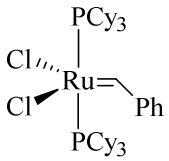
## DISCUSSION AND RESULTS

**Figure 1:** Ruthenium based catalysts used for controlled polymerization of Pegylated ROM monomers




Grubbs 3rd Generation Catalyst

GC3

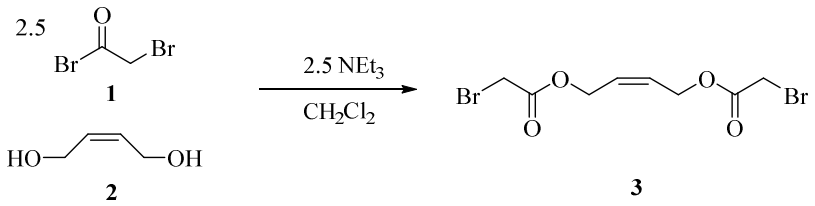



Grubbs 1st Generation Catalyst

GC1

The two catalyst shown in Figure 1 are Grubbs 1st (**GC1**) and 3rd (**GC3**) generation-type ruthenium-alkylidene complexes used for controlled Ring Opening Metathesis Polymerization (ROMP) due to their ability to produce ROMPolymers with high molecular weight control. The catalyst is available in large quantities and was developed in the Schanz laboratories[12]. Both catalysts are inert towards sensitive functional groups while being highly active. Both catalysts are capable of controlled ROMP (the molecular weight can be controlled) based on their fast initiation rates. (7-oxa)norbornene derivatives[9-13] are very good monomers for this project because of the low tendency to engage in secondary metathesis reactions such as internal chain transfer via ring closing metathesis;[14-17] they are highly capable monomers for controlled ROMP due to their high ring strain. We are confident that the combination of monomers and catalysts will enable us to produce multifunctional polymers to attach covalently to cell free hemoglobin.

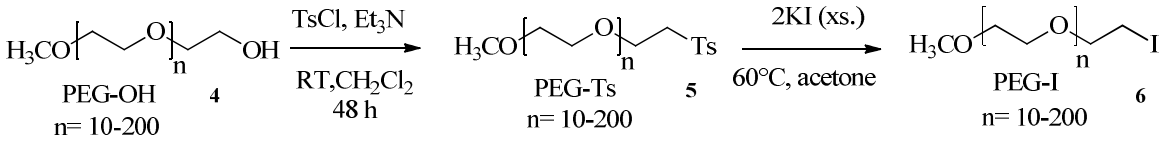
The first generation catalyst **GC1** is a much slower propagator than the third generation catalyst **GC3.** Hence, ROMP will require longer polymerization time; however, **GCI** is less prone to decompose when conditions are not ideal. **GC3** is a faster propagator, but tends to decompose under conditions that are not ideal. The overall advantage of using these catalysts is the ability to control the molecular weight due to their fast initiation compared to the propagation. **GC1** and **GC3** are very efficient initiators, so all of the polymer chains should be about the same length under the assumption that the rate of propagation is stable.




**Scheme 1**. Synthesis of Chain Transfer Agent for Termination of ROMP

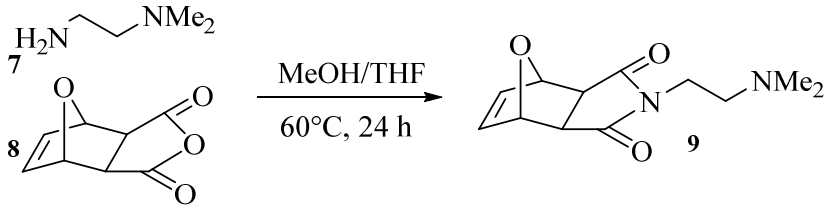
ROMP requires a terminating agent to end the polymerization; otherwise, the polymerization will continue and cross linking can occur. Bromoacetyl Bromide **1** reacted with the Z-diol **2** with base to yield diester **3.** This product can, when added, attach to the end of the polymer chain while terminating the polymerization. The attached bromo end group can make the covalent link to the lysine amino group of the hemoglobin.

*Synthesis of Cationic, Water Soluble Monomer species.*



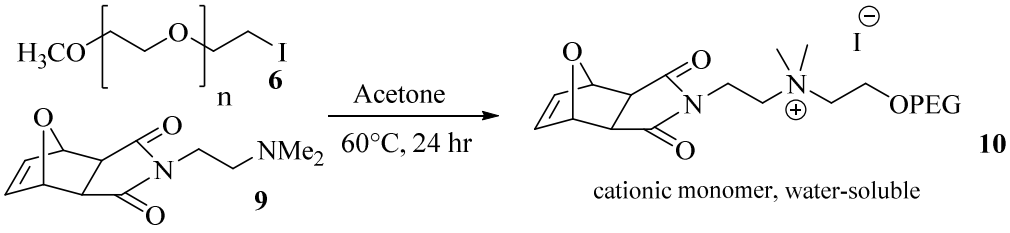

**Scheme 2.** Synthesis of Polyethylene Glycol Iodide using a series of nucleophilic substitution reactions.

For our first monomer, we started with commercially available compound **4**, polyethylene glycol (PEG). We synthesized the iodide via the tosylate intermediate **5** in overall 82.5% yield. The PEG-Iodide **6** was produced at 67.5% yield.




**Scheme 3.** Synthesis of Dimethylamino Imide monomer from Carboxylic Anhydride

7-oxanorbornene derivative **9** was synthesized with an ancillary amino group. Ethylene Diamine **7** reacted with the carboxylic anhydride **8** (produced in a known reaction[10]according to Scheme 3) to obtain the imide monomer **9**.

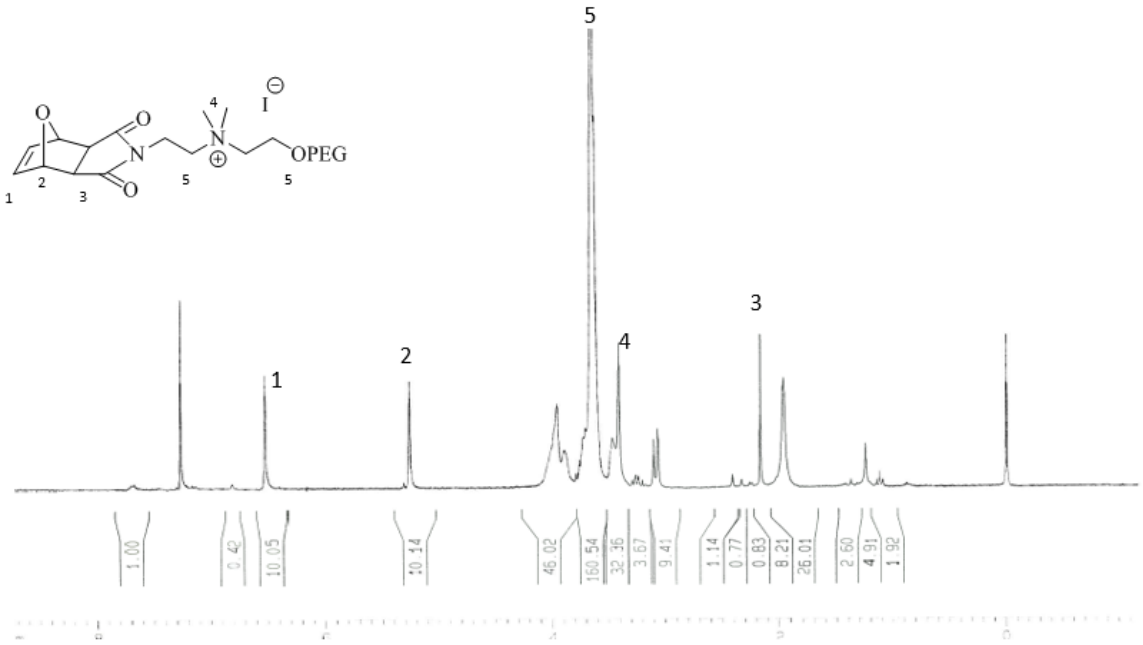



**Scheme 4.** Synthesis of Cationic, PEG-containing Exo-7-oxanorbornene Derivative

The cationic monomer **10** is produced by the addition of PEG-Iodide **6** to the di-methyl amino group in monomer **9**. This monomer has a counter iodide anion. In the context of ROMP, the iodides can substitute the chlorines on the ruthenium catalyst..

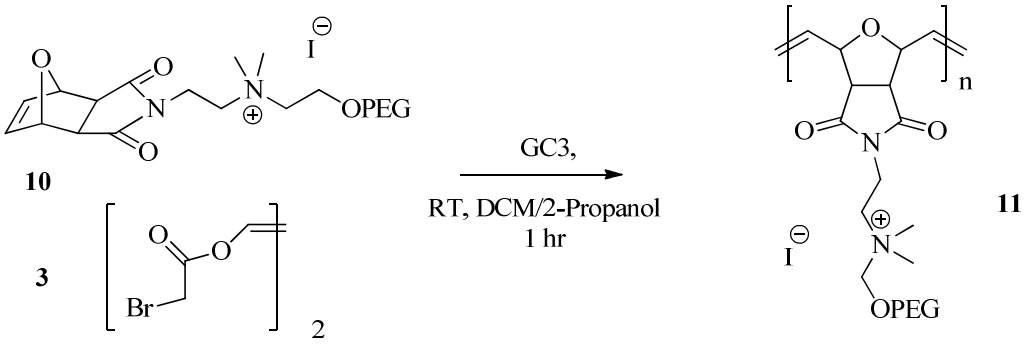
Iodide is a larger ligand than chloride. This has two consequences. The larger iodide ligands cause the initiation stage of the catalyst to accelerate. The larger ligands destabilizes the leaving ligand at the catalyst due to steric hinderance. However, this steric hindrance also slows the propagation stage. These larger ligands can hinder the swing through mechanism that controls the propagation. Overall, the iodide causes faster initiation which helps have a more controlled molecular weight, but causes faster catalyst degradation which is linked to the initiation rates.

**Figure 2:** $^{1}$H NMR of cationic PEG-monomer, 250 MHz, $CDCl_3$




The $^{1}$H NMR spectrum of the water soluble, cationic monomer **9** (Fig.2) exhibits its furthest downfield peak (1) for the two hydrogen atoms bonded to the alkene in the bicyclic ring system. The second farthest downfield peak (2) is the signal for the two hydrogen atoms on the oxo-bridged carbon atoms. The largest peak (5) includes the protons that make up the PEG chain and overlap with the protons between the nitrogen atoms. Peak 4 consists of the protons on the two equivalent methyl groups on the

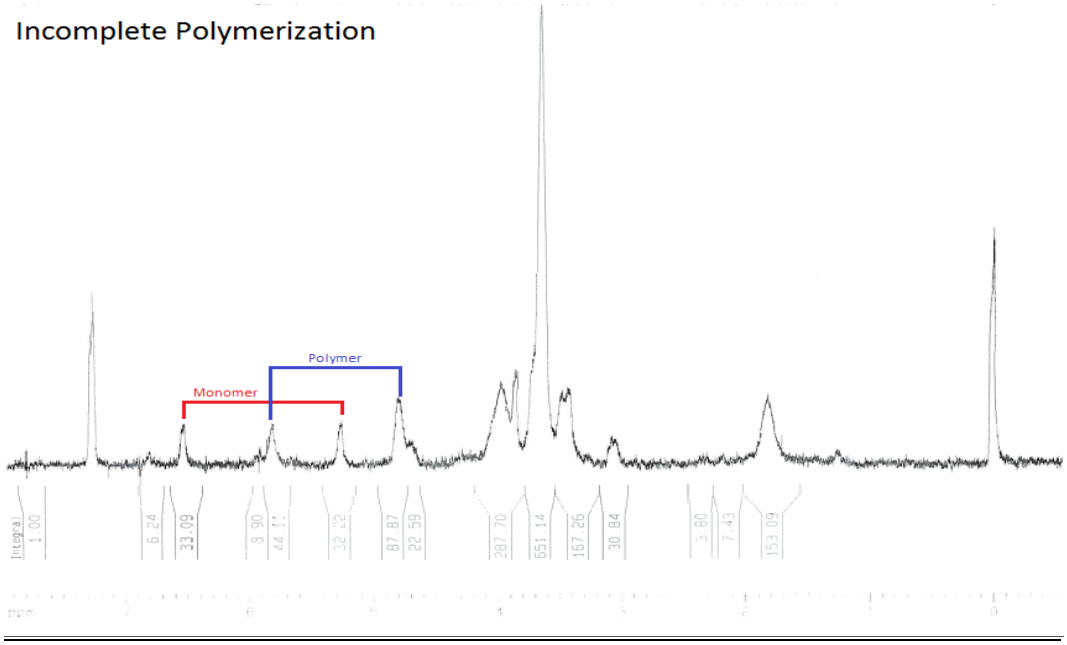
ammonium nitrogen atoms. The ratio of 2:32 between hydrogen atoms (1) and (5) is indicative of 16 $CH_2$ groups in the PEG, the expected amount for a PEG with the molecular weight of 400 g/mol as used in this monomer.




**Scheme 5**. ROMPolymerization of cationic-monomer, 5% catalyst loading with 3rd generation catalyst

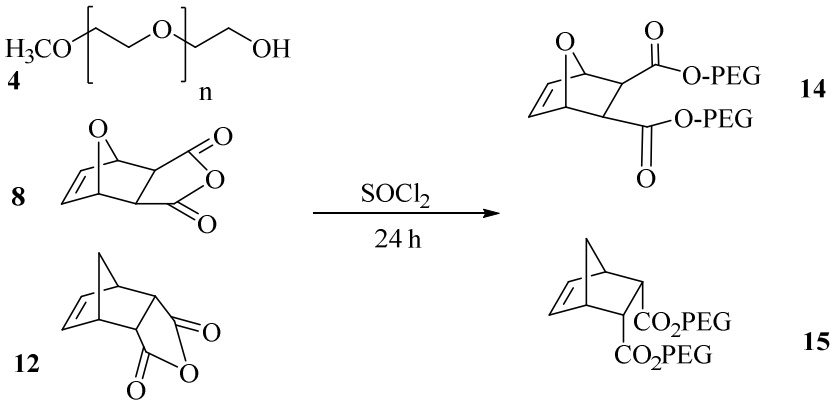
Monomer **10** was polymerized Grubbs 3rd generation catalyst **GC3**. The reaction took place under inert gas in methylene chloride. With 5% catalyst loading, polymerization ran for one hour, and chain transfer reagent **3** added after the hour (Scheme 5).

**Figure 3:** $^{1}$H NMR spectrum ($CDCl_3$, 250 MHz) of the polymerization mixture after addition of terminating agent and removal of solvent.




The $^{1}$H NMR spectrum (Figure 3), indicates an incomplete polymerization (~75%) by exhibiting signals for both monomer and polymer at with integrations of 1:3 monomer to polymer. There are a few possibilities as to why there was incomplete conversion. The concentration of the iodide may have inhibited the reaction from going to completion; iodide is known to reduce propagation rates meaning the one hour reaction time may have been too short. Another possibility is that the ruthenium catalyst has degraded in polar solvent needed for the cationic monomer. In this case, longer reaction times will not change conversion and a low molecular weight control will also be observed. Due to the unexpected problems with the cationic monomer **10**, we decided to shift our research focus to making neutral pegylated monomers.

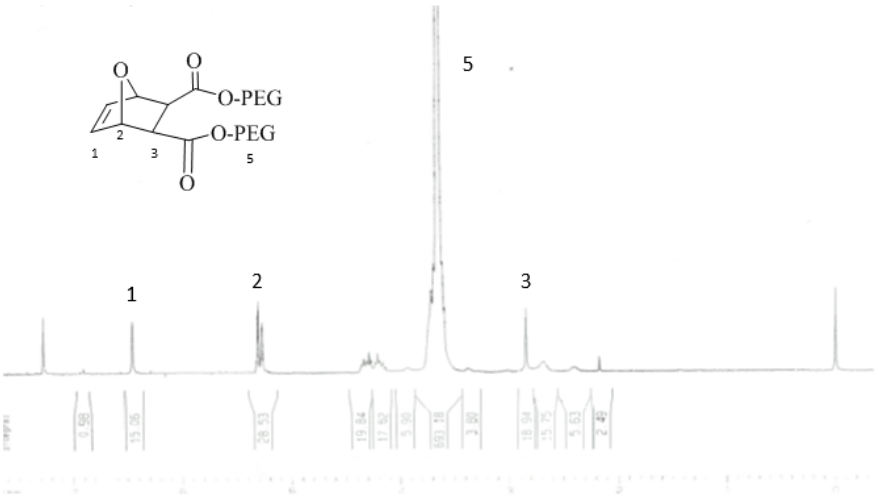
*Synthesis of Neutrally Charged Monomer Species.*



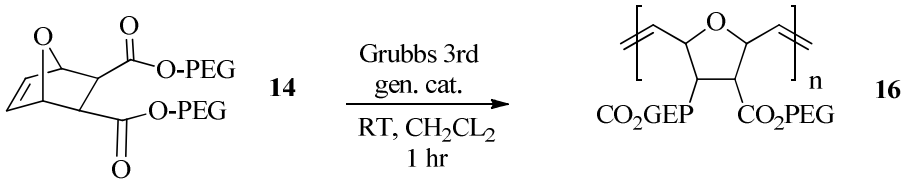

**Scheme 6.** Direct esterification of (7-oxa)-norbornene precursors resulting in neutrally charged diester monomers

The first neutrally charged pegylated monomer **7** is produced from the direct esterification of the carboxylic anhydride **3** with polyethylene glycol **4**. The reaction requires no solvent; thionyl chloride (0.5 mL) is added and the solution stirred for 24 hours. Over the 24 hours, the solution turns grey in color. The workup involves an extraction with methylene chloride and bicarbonate solution to remove excess PEG-OH.

**Figure 4:** $^{1}$H NMR spectrum ($CDCl_3$, 250 MHz) of the7- oxa-norbornene diester monomer



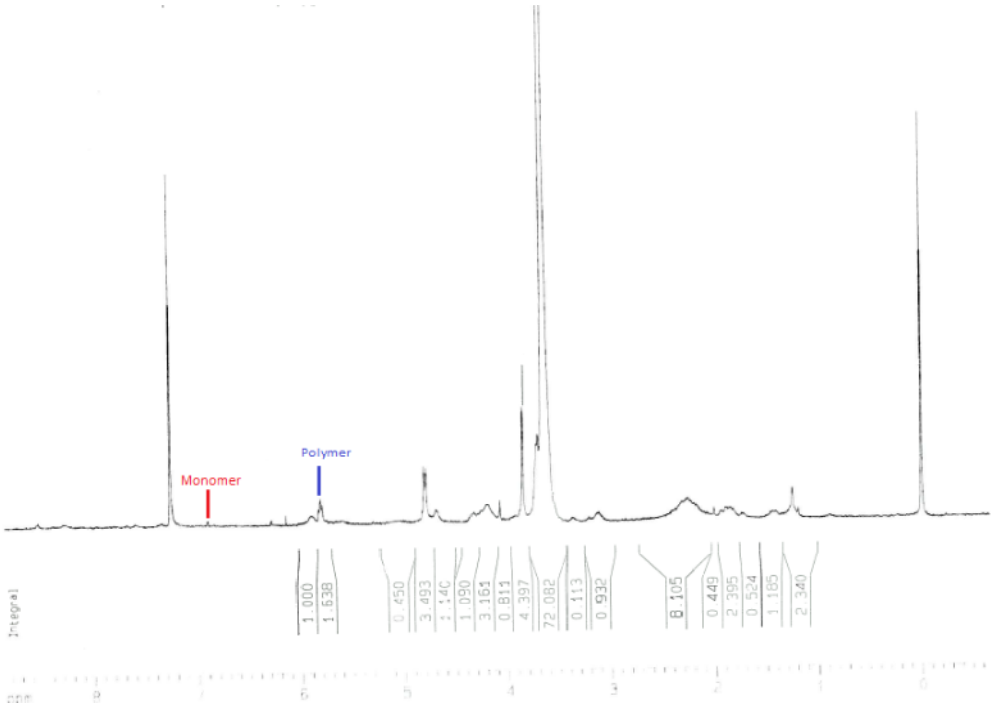
The $^{1}$H NMR spectrum of 7 exhibits the expected signals 1,2,3 for the (oxa)norbornene fragment and 5 for the PEG; the integration of 1:40 is approximately the expected ratio of 2:64 to but may also indicate some PEG-OH contamination in the monomer.




**Scheme 7.** Synthesis of ROMP homopolymer bearing PEG

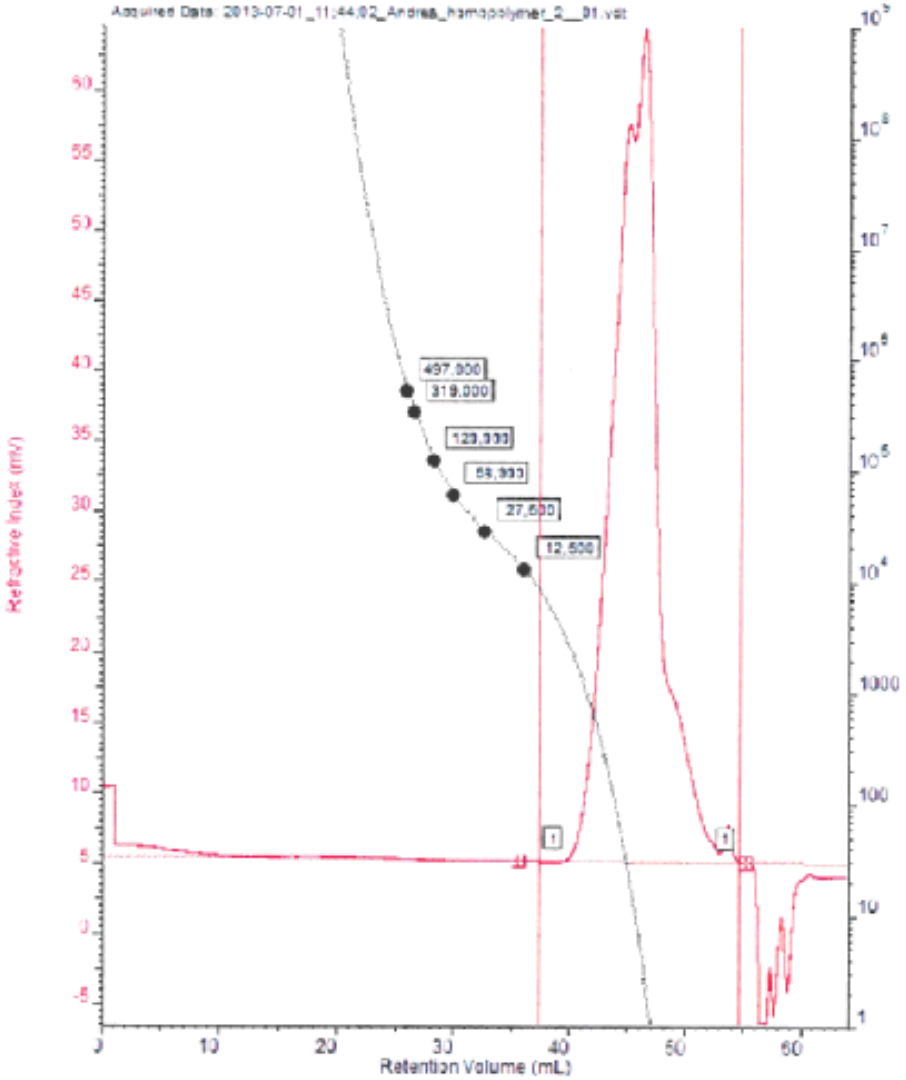
The ROMPolymerization (Scheme 7) of monomer **14** was performed in methylene chloride and 5% catalyst loading. The solution is mixed for an hour. The terminating agent (ethyl vinyl ether) was added and the solvent is removed.

**Figure 5:** $^{1}$H NMR spectrum ($CDCl_3$, 250 MHz) of the ROMPolymerization of 7- oxa-norbornene diester monomer

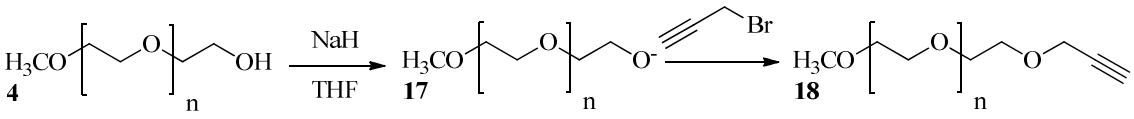


This polymerization appeared to have reached a high conversion based on the $^{1}H$ spectrum (Fig. 4). The monomeric peak is virtually nonexistent. The polymer peak is clearly defined. For further confirmation of the homopolymer, gel permeation chromatography (GPC) is used. The purpose of the GPC is to determine the molecular weight and molecular weight distribution. Due to the use of Grubbs 1st and 3rd catalyst which is capable of controlled ROMP, the molecular weight distribution should be relatively narrow. The polymer has a theoretical molecular weight 19,000 g/mol.

**Figure 5:** GPC analysis of PEG- diester Homopolymer

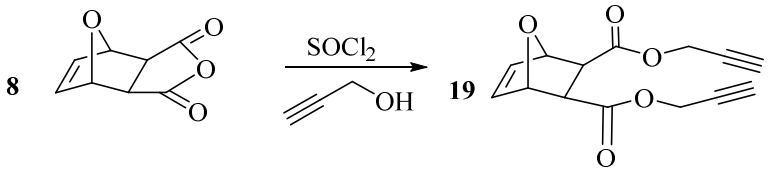


The data given by the GPC tells us that the "polymer" has a molecular weight distribution significantly smaller than 12,500g/mol. This suggests the presence of an oligomer (a few repeating units) instead of a polymer. There are two reasons for low degrees of polymerization. Chain transfer would cause an increased number of polymer chains growing causing a decrease in the average molecular weight. The polymerization may have just needed more time to complete the polymerization.



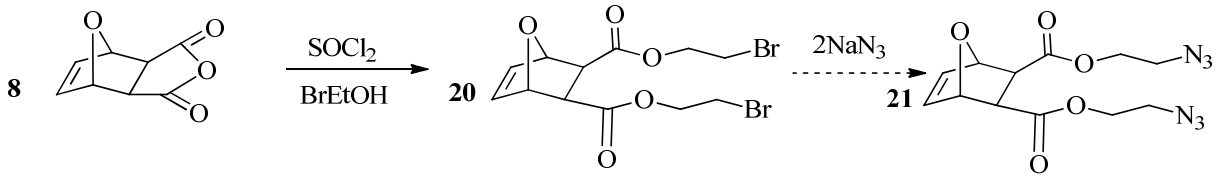

**Scheme 8.** Synthesis of Propargyl PEG via reduction and addition

PEG **4** was deprotonated yielding the intermediate **17**. Addition of propargyl bromide renders propargyl PEG **18**. Propargyl PEG **18** has a reactive alkynyl group, which can be used for post polymerization modification.




**Scheme 9.** Synthesis of Neutrally Charged alkyne diester monomer

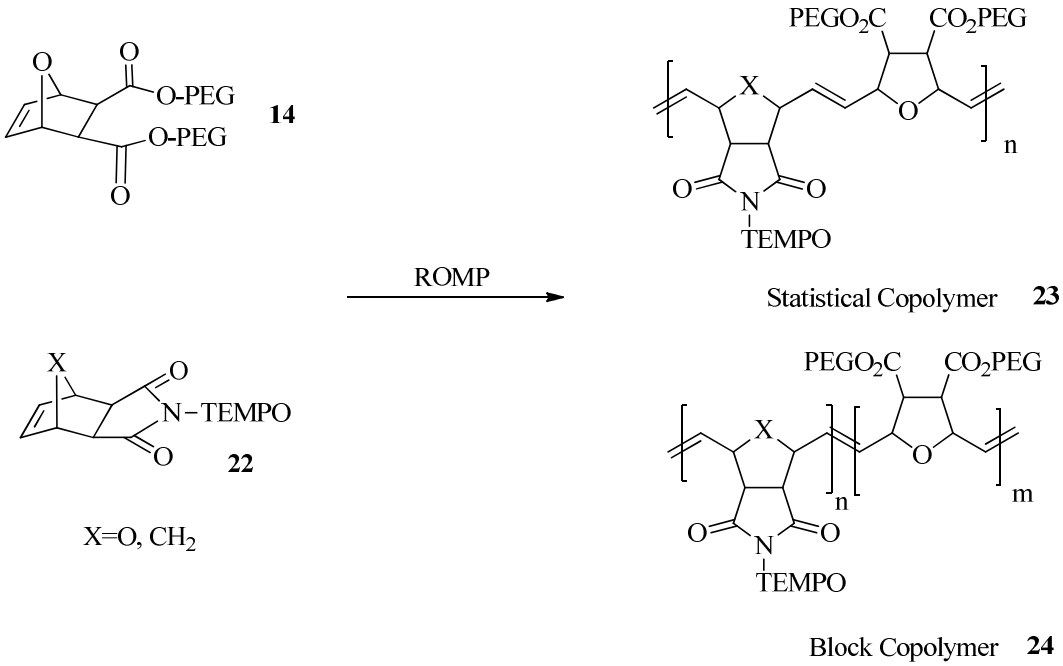
The exo-carboxylic anhydride **8** was reacted with propargyl alcohol yielding monomer **19**. The thionyl chloride opens the bicyclic ring making a diester. We intend to modify this monomer via click reaction with respective functional azides. The modifications have not been started as of yet.




**Scheme 10.** Synthesis of Neutrally Charged Bromo diester monomer

In similar fashion, exo-carboxylic anhydride **8** was reacted with bromoethanol yielding monomer **20**. The thionyl chloride opens the bicyclic ring making a diester. This monomer can be modified by converting it into the diazide monomer **21**. This would be the complementary approach to the "click" introduction of the functional groups.

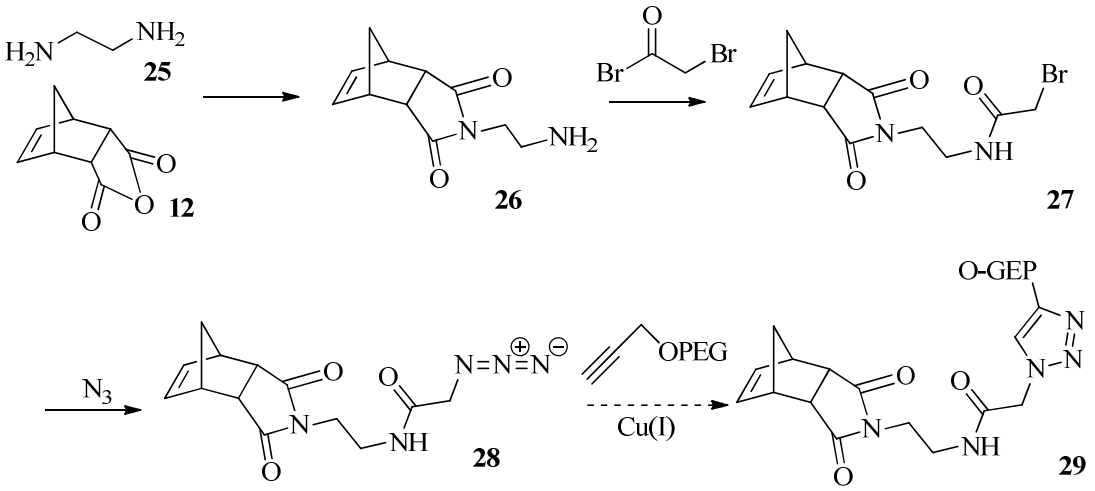
*Future Outlook*




**Scheme 11.** Planned Synthesis of ROMP copolymers bearing PEG and TEMPO groups

The ultimate goal of the research is to make copolymers containing PEG and TEMPO. Polymerization of neutral PEG and TEMPO monomers would yield the copolymers **23** and **24** (Scheme 11). The copolymerization of two monomeric units, pegylated diester **14**

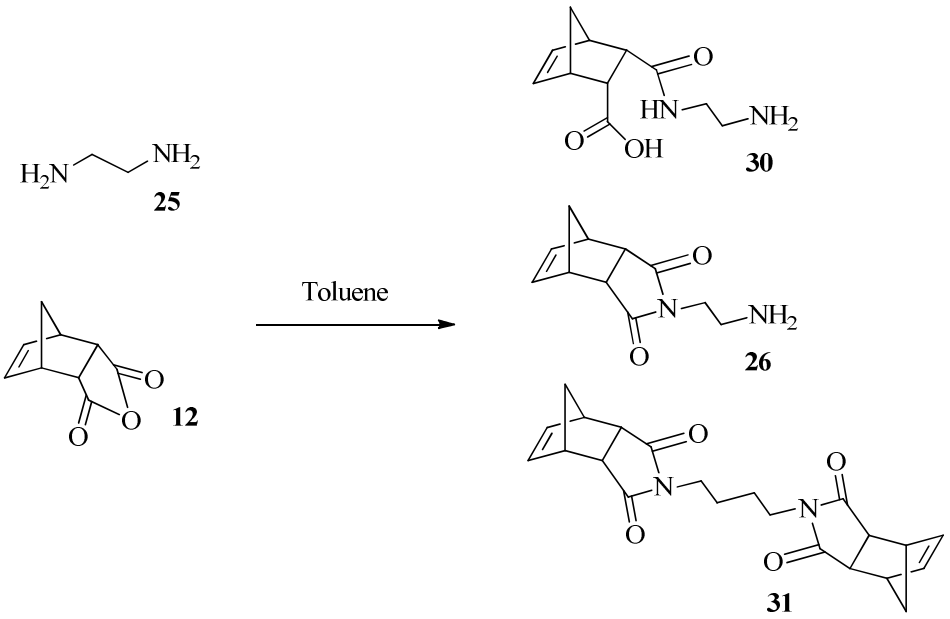
and TEMPO monomer **22**, can yield two different types of polymers. The first is statistical copolymer **23**. This polymer is achieved by polymerizing monomers **14** and **22** at the same time, meaning you get a polymer with a statistical mix of TEMPO and PEG monomers. The block copolymer **24** is achieved by sequentially polymerizing monomer **14** and adding monomer **22** after all of monomer **14** is polymerized. This polymer should have literally a block of polymer consisting of only diester monomeric units and a block consisting of only TEMPO monomeric units. Unfortunately, these polymerizations were attempted twice and were unsuccessful in both cases.




**Scheme 12.** Proposed synthesis of endo-norbornene pegylated triazole monomer

The synthesis laid out in Scheme 12 is a proposed multistep procedure to generate a monomer **28** that can be modified to incorporate PEG i.e. monomer **2** or other functionalities. Ethylene Diamine **25** will aminate the anhydride to produce monomer **26**. The addition of bromoacetyl bromide renders **27**. The bromine acts as a good leaving group allowing the addition of the azide. PEG can be incorporated via click reaction using a copper catalyst to change an azide to a triazole. The advantage of using the click

reaction is that it is a well-known, very reliable and quantitative reaction that forms no additional side products. Click is also a very fast reaction that uses an inexpensive Cu(I) catalyst and is not sensitive to functional groups. Propargyl PEG **18** could be attached to monomer **28** either before or after polymerization to render the Pegylated monomer **29** or the corresponding polymer.




**Scheme 13.** Amination of Endo-norbornene anhydride through four different conditions

The imide monomer **26** was initially thought to be isolated; however, a dimer **31** was formed. However, monomer **26** is observed in the NMR. Current work is being done to isolate product **26** and form a protocol. For the synthesis, ethylene diamine solution is toluene is made; the anhydride is added slowly while heating the solution. The solution needs to be gently heated for 24 hours, then phosphoric acid is added and the solution refluxed for 72 hours. The reaction needs to be monitored to find when our product of interest is formed. If the reaction is not given enough time to react, then the unsymmetric

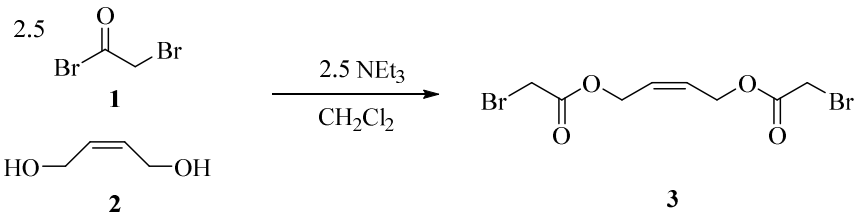
amide product **30** is dominant. If the reaction goes for too long, then a dimer **31** is formed. Isolation of monomer **26** is crucial.

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Hans Schanz for mentoring me and helping build my skills as a chemist during my undergraduate career. His guidance has aided me throughout my time of research and writing of this thesis. He encouraged me to push myself to see what I am truly capable of. Dr. Schanz has helped me see my potential, and for that, I am eternally grateful. I would like to thank Dr. Don McLemore for aid in analysis of polymers via GPC and teaching me more about the instrumentation. I would like to thank Dr. Jeff Orvis for his in depth explanations of numerous NMR techniques.

I thank my fellow labmates in the Schanz Research Group: Morgan Mullens, Cameron Feriante, Koomi Orr, Jessica Grey, and Inez Parker for all the fascinating conversations and interesting situations we have experienced in the last two and a half years. I want to thank the College Office of Undergraduate Research (COUR) for a research stipend, and the Interdisciplinary Pilot Funds (College of Science and Technology) for the initial funds for the project.

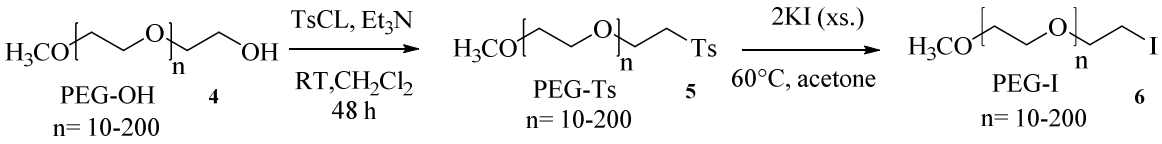
EXPERIMENTAL




**Scheme 1**. Synthesis of Chain Transfer Agent for Termination of ROMP

**Diester 3:**

Cis-2-Butene-1,4-diol **2** (0.3397g) was measured into a vial. Triethylamine ($NEt_3$) (1.0027g) was measured into a separate vial. Methylene chloride (20 mL) was poured into a round bottom flask. The flask as placed in an ice bath; $NEt_3$ and **2** were quickly pipetted into the chilled flask; the flask was sealed. The Bromoacetyl Bromide **1** was measured into a vial in the nitrogen dry box and removed from the inert gas atmosphere. Compound **1** was added to the flask and stirred for 1 hour. After 1 hour, an extraction was done. The first wash was with (0.1 M) phosphoric acid to remove excess Triethylamine. The second wash was with a (0.1M) bicarbonate solution to neutralize any acid left over. The organic phase was dried over sodium sulfate and the solvent removed. To purify the compound, a flash column was used. Ethyl acetate was used as the running solvent. The solvent of the filtrate was removed and dried in the vacuum oven.



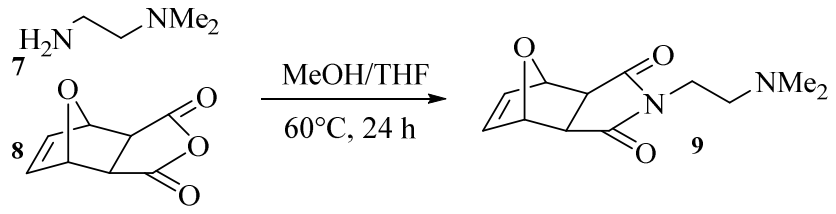

**Scheme 2.** Synthesis of Polyethylene Glycol Iodide using a series of nucleophilic substitution reactions.

**PEG-Tosylate 5:**

10 grams of Polyethylene Glycol (PEG-400) **4** was added to a 500mL round bottom flask. Methylene chloride was added as the solvent. 1 molar equivalent of tosyl chloride (4.766 g) was added next. The solution stirred for 5 minutes to dissolve the starter. Last, 1 molar equivalent of Triethylamine (0.303g) was added; the solution was stirred for 6 days. An extraction was done as a workup. The first wash was with (0.1 M) phosphoric acid. The second wash was with a (0.1M) bicarbonate solution; the separation settled for 1 hour. The last wash was with water. The organic phase was separated, dried over sodium sulfate and the solvent removed. The total yield for the tosylate intermediate **5** was 82. 5%.

**PEG-Iodide 6:**

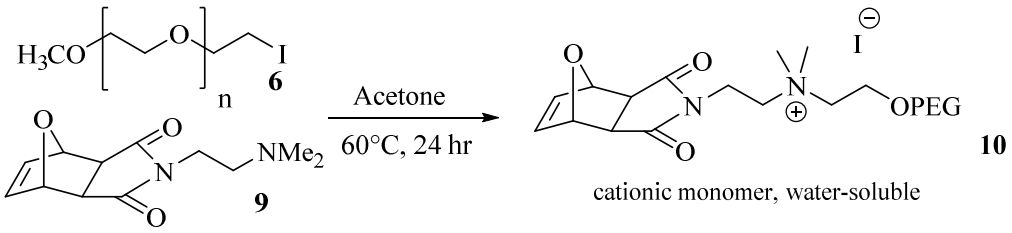
PEG-tosylate **5** (12.372 g) was dissolved in acetone (40 mL). 2 molar equivalents of Potassium Iodide was added to the solution and stirred. Within the first 30 minutes of stirring, a solid formed. More acetone was added and the slurry was refluxed overnight. The refluxed slurry was filtered removing excess potassium iodide. The filtrate was a deep red. The solvent was replaced with DCM and an extraction was done. The first wash was with (0.1M) sodium sulfate and (0.1M) bicarbonate solution. The organic phase was greatly decolorized producing a light yellow. The yield for producing PEG –Iodide **6** was 67.5%.




**Scheme 3.** Synthesis of Dimethylamino Imide monomer from Carboxylic Anhydride

**Diemiethylamino Imide 9:**

Exo-3,6-ep-oxy-1,2,3,6-tetrahydrophtalic anhydride **8** was added to a round bottom flask in 1:1 MeOH/THF. The solution was stirred at 50°C; the N,N- Dimethyl ethylenediamine **7** was added slowly to the solution. The solution stirred overnight. The solvent was removed and replaced with MeOH. The flask was placed in the freezer to yield white crystals. To get the remaining product, the methanol was removed and dried in the vacuum oven. Ethanol (50 mL) and concentrated HCl (2mL) was added. Once the precipitate was dissolved, the solution was removed to yield monomer **9**.

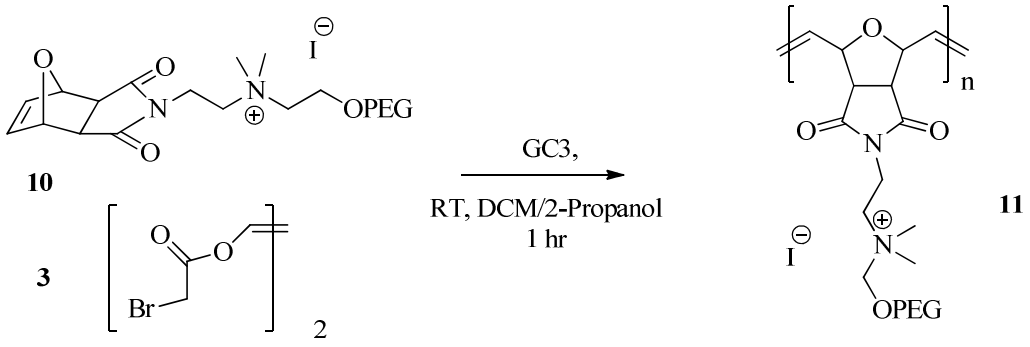



**Scheme 4**. Synthesis of Cationic, PEG-containing Exo-7-oxanorbornene Derivative

**Cationic, Water Soluble 10:**

PEG-Iodide **6** (0.3797g) and monomer **9** (0.1758 g) were added to a round bottom flask with acetone (20 mL) and refluxed for 3 hours. The reaction was incomplete. The solution was refluxed for an additional 4 days. An extraction was done; the first wash was

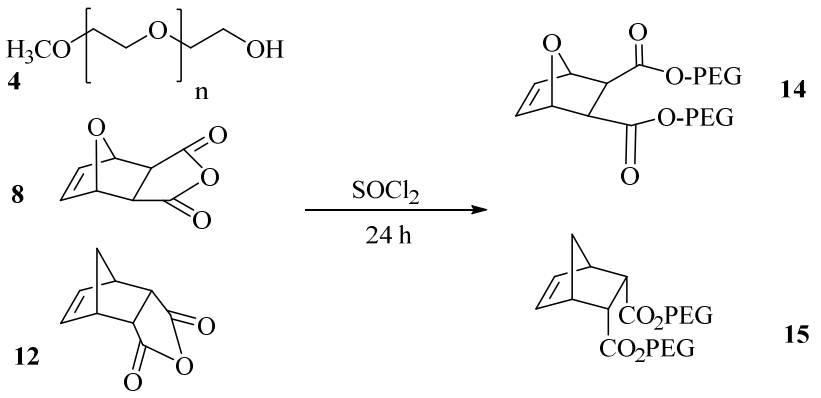
DCM/water. The second wash was with sodium thiosulfate. The last wash was with water. The organic phase was dried over sodium sulfate yielding cationic monomer **10**.




**Scheme 5**. ROMPolymerization of cationic-monomer, 5% catalyst loading with 3rd generation catalyst

## Cationic Polymer 11:

The PEGI-monomer **10** (0.3508 g) was added to a round bottom flask and placed in the nitrogen dry box. The Grubbs third generation catalyst GC3 was added, and the solution stirred for 30 minutes. The terminating agent Bromo-diester **3** was added; the solution stirred for an hour. The solvent was removed and the product was dried.



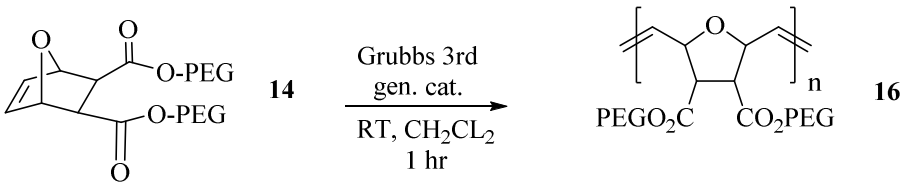

**Scheme 6.** Direct esterification of (7-oxa)-norbornene precursors resulting in neutrally charged diester monomers

**Diester Pegylated Norbornene derivative 15:**

PEG-400 **4** (5g) was weighed into a round bottom flask. The carbic anhydride **12** was weighed and placed in the flask. Thionyl chloride (3 mL) was added and the flask stirred for 7 days. After 7 days, an extraction was done in two parts. The first wash was with bicarbonate solution to remove acid produced during the reaction. The second wash was with water. The bicarbonate wash separated for 1.5 hours. The second wash settled for about 12 hours. Sodium chloride was added to each wash to expedite the process of separation. The organic phase was dried over sodium sulfate and the solvent removed yielding our product **15**.

**Diester Pegylated (7-oxa)norbornene derivative 14:**

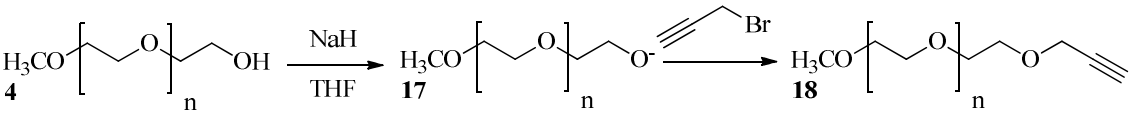
PEG-400 1 (5g) was weighed into a round bottom flask. The carboxylic anhydride **8** was weighed and placed in the flask. Thionyl chloride (3 mL) was added and the flask stirred for 7 days. After 7 days, an extraction was done in two parts. The first wash was with bicarbonate solution and the second wash was with water. The bicarbonate wash separated for 1.5 hours. The second wash settled for about 12 hours. Sodium chloride was added to each wash to expedite the process of separation. The organic phase was dried over sodium sulfate and the solvent removed. Yielding our product **14**.




**Scheme 7.** Synthesis of ROMP homopolymer bearing PEG

**Neutrally Charged Polymer 16:**

The pegylated diester monomer **14** was weighed into a vial and placed in the nitrogen dry box. The catalyst was weighed into a small round bottom flask; 15 mL of methylene chloride was added and the monomer **14** was added last. The solution polymerized for an hour before the terminating agent (ethyl vinyl ether) was added. The purification required a filtration. The methylene chloride was replaced by methanol. Activated carbon was added to the solution and settled for 10 minutes. After that, the solution was filtered through celite. A colorless filtrate was produced. The solvent was removed and dried in the vacuum oven yielding polymer **16**.

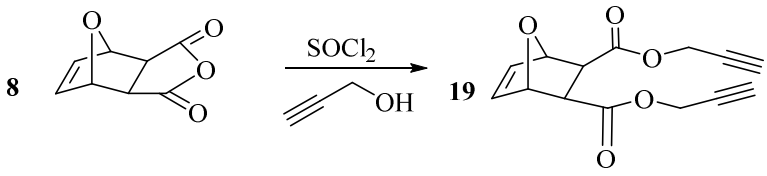



**Scheme 8.** Synthesis of Propargyl PEG via reduction and addition

**Propargyl PEG 18:**

PEG **4** was weighed into a round bottom flask and brought into the nitrogen dry box. The solvent tetrahydrofuran (THF) was added to the flask; the flask was removed from the dry box. 1.5 molar equivalents of Sodium Hydride (60% dispersed in mineral oil) was added to the solution. A condenser and bubbler were added to the apparatus; the solution stirred for 24 hours. The intermediate PEG-hydride **17** was formed. 1.3 molar equivalents of propargyl bromide (80% in toluene) was added to **17** and the solution

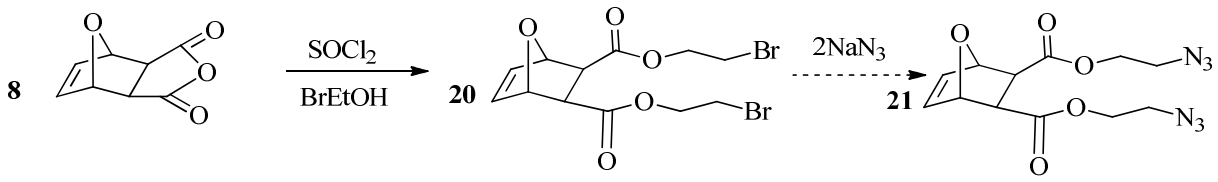
refluxed for 4 days. The THF was replaced by methylene chloride. An extraction was done with 3 water washes.




**Scheme 9.** Synthesis of Neutrally Charged alkyne diester monomer

**Diester Alkyne 19:**

Exo-carboxylic anhydride **8** and propargyl alcohol were added to a round bottom flask in a 1:5 molar ratio of anhydride to alcohol. Thionyl chloride was added and the solution stirred for 24 hours. An extraction was done in three parts. The first wash was with water/DCM. The second wash was with bicarbonate solution. For the third wash, the organic solvent was replaced with ethyl acetate. The organic phase was dried over sodium sulfate and the solvent replaced by 1:5 cyclohexane to t-butyl-methyl ether. The solution was placed in the freezer to recrystallize. The liquid was decanted off yielding pure white crystals of **19.**




**Scheme 10.** Synthesis of Neutrally Charged Bromo diester monomer

**Bromo Diester 20:**

Exo-carboxylic anhydride **8** and bromoethanol were added to a round bottom flask in a 1:5 molar ratio of anhydride to alcohol. Thionyl chloride was added and the solution stirred for 24 hours. An extraction was done in three parts. The first wash was with water/DCM. The second wash was with bicarbonate solution. For the third wash, the organic solvent was replaced with ethyl acetate. The organic phase was dried over sodium sulfate and the solvent replaced by t-butyl-methyl ether. The solution was placed in the freezer. A precipitate formed and was filtered off of product **20.**

REFERENCES


1. Hsia, C.J.C., Ma, L.: "A Hemoglobin-Based Multifunctional Therapeutic: Polynitroxylated Pegylated Hemoglobin," *Artif. Organs* **2012**, *2*, 215-220**Ma**, L.; et. al.; *J. Lumin*. **2011**, *131*, 461–464.
2. Walson, K.H., M. Tang, M., Glumac, A. Alexander, H., Manole, M.D., Ma, L., Hsia, C.J.C., Clark, R.S.B., Kochanek, P.M., Kagan, V.E., Bayır, H.: "Normoxic Versus Hyperoxic Resuscitation in Pediatric Asphyxia Cardiac Arrest: Effects on Oxidative Stress," *Crit. Care Med*. **2011**, *39*, 335-343.
3. Shellington, D.K., Du, L., Wu, X., J. Exo, J.L., Vagni, V.A., Ma, L., Janesko-Feldman, K., Clark, R.S.B., Bayır, H., Dixon, C.E., Jenkins, L.W., Hsia, C.J.C. Kochanek, P.M.: "Polynitroxylated Pegylated Hemoglobin: A Novel Neuroprotective Hemoglobin for Acute Volume-Limited Fluid Resuscitation after Combined Traumatic Brain Injury and Hemorrhagic Hypotension in Mice," *Crit. Care Med*. **2011**, *39*, 494-505.
4. Stoyanovsky, D.A., Kapralov, A., Huang, Z., Maeda, A. Osipov, A., Hsia, C.J.C., Ma, L., Kochanek, P.M., Bayr, H., Kagan, V.E.: "Unusual Peroxidase Activity of Polynitroxylated Pegylated Hemoglobin: Elimination of $H_2O_2$ Coupled with Intramolecular Oxidation of Nitroxides," *Biochem. Biophys. Res. Commun*. **2010**, *399*, 139-143.
5. Exo, J.L., Shellington, D.K., Bayır, H., Vagni, V.A., Janesco-Feldman, K., Ma, L., Hsia, C.J.C., Clark, R.S.B., Jenkins, L.W., Dixon, C.E., Kochanek, P.M.: "Resuscitation of Traumatic Brain Injury and Hemorrhagic Shock with Polynitroxylated Albumin, Hextend, Hypertonic Saline, and Lactated Ringer's:

Effects on Acute Hemodynamics, Survival, and Neuronal Death in Mice," *J. Neurotrauma* **2009**, *26*, 2403–2408.

6. Brockman, E.C., Bayır, H., Blasiole, B., Shein, S.L., Fink, E.L., Dixon, C., Vagni, V.A., Ma, L., Hsia, C.J.C., Tisherman, S.A., Kochanek, P. M.: "Polynitroxylated-Pegylated Hemoglobin Attenuates Fluid Requirements and Brain Edema in Combined Traumatic Brain Injury Plus Hemorrhagic Shock in Mice" *Journal of Cerebral Blood Flow & Metabolism* **2013**, *33*, 1457-1464.
7. Grubbs, R.H. (*Eds*.): "Applications in Polymer Synthesis" in *Handbook of Metathesis,* Wiley-VCH, Weinheim, **2003**, *Vol. 3*Bielawski, C.; Grubbs,R; *Prog. Polym. Sci.* **2007**, *32*, 1-29.
8. Grubbs, R.H.: "Olefin Metathesis" *Tetrahedron* **2004**, *60*, 7117-7140.
9. Dunbar, M., Balof, S.L., LaBeaud, L.J., Yu, B., Lowe, A.B., Valente, E.J., Schanz, H.-J.: "Improved Molecular Weight Control in Ring Opening Metathesis Polymerization (ROMP) Reactions with Ru-Based Olefin Metathesis Catalysts Using N-Donors and Acid: A Kinetic and Mechanistic Investigation" *Chem. Eur. J.* **2009**,*15*, 12435-12446.
10. Rankin, D., P'Pool, S.J., Schanz, H.-J., Lowe, A.B.: "The Controlled Homogeneous Organic Solution Polymerization of New Hydrophilic Cationic exo-7-Oxanorbornenes via ROMP with RuCl2(PCy3)2CHPh in a Novel 2,2,2-Trifluoroethanol/Methylene Chloride Solvent Mixture" J. Polym. Sci. A, Polym. Chem. 2007, 45, 2113-2128.

11. Balof, S.L.: “Ruthenium Based Olefin Metathesis Catalysts Bearing pH-Responsive Ligands: External Control of Catalyst Solubility and Activity” Dissertation, The University of Southern Mississippi 2011.
12. Balof, S.L., Yu, B., Lowe, A.B., Ling, Y., Zhang, Y., Schanz, H.-J.: “Ru-Based Olefin Metathesis Catalysts Bearing pH-responsive N-Heterocyclic Carbene (NHC) Ligands: Activity Control via Degree of Protonation” Eur. J. Inorg. Chem. 2009, 1717-1722.
13. Dunbar, M.A., Balof, S.L., Roberts, A.N., Valente, E.J., Schanz, H.-J.: “pH-Responsive Ruthenium-Based Olefin Metathesis Catalysts: Controlled Ring-Opening Metathesis Polymerization in Alcoholic and Aqueous Media upon Acid Addition” Organometallics 2011, 30, 199-203.
14. Hong, S.H., Sanders, D.P., Lee, C.W., Grubbs, R.H.: “Prevention of Undesirable Isomerization during Olefin Metathesis” J. Am. Chem. Soc. 2005, 127, 17160-17161.
15. Schmidt, B.: “Ruthenium-Catalyzed Olefin Metathesis Double-Bond Isomerization Sequence” J. Org. Chem. 2004, 69, 7672-7687.
16. Fürstner, A., Thiel, O.R., Ackermann, L., Schanz, H.-J., Nolan, S.P.: “Ruthenium Carbene Complexes with N,N'-Bis(mesityl)imidazol-2-ylidene Ligands: RCM Catalysts of Extended Scope”, J. Org. Chem. 2000, 65, 2204-2207.
17. Courchay, F.C., Sworen, J.C., Ghiviriga, I., Abboud, K.A., Wagener, K.B.: “Understanding Structural Isomerization during the Ruthenium-Catalyzed Olefin Metathesis: A Deuterium Labeling Study” Organometallics 2006, 25, 6074-6086.